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CHROMOSOME ENDS IN *DATURA PRUINOSA*

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In the present paper we are adding *D. pruinosa* to the list of those species of *Datura*, the chromosomes of which have been given numerical representation in terms of prime type 1 (PT1),¹ our tester race in *D. stramonium*. *D. pruinosa* is a Mexican species which belongs to the Dutra² section of the genus. The tester race came from Oaxaca; other races came from the adjacent state of Puebla.

By our method of examining chromosomes at the first meiotic metaphase in pollen-mother-cells, we usually detect only those changes which have occurred in the arrangements of the ends of chromosomes,³ as a result of segmental interchange between non-homologous chromosomes. It has taken six years to complete the identification of the chromosomes of *D. pruinosa* because, with the exception of *D. leichhardtii*, it could not be crossed with any other species in either the Stramonium or Dutra sections of the genus. Before any other crosses could be made, strains of *leichhardtii* had to be developed which included in their chromosome complement the end arrangements of the four *pruinosa* chromosomes which are different from the tester race of *leichhardtii*.⁴ Even with such strains, however, no cross could be made with the tester race of *stramonium* but only with extracted lines.

There were repeated instances of incompatibilities when we attempted to obtain plants which resembled *stramonium* in morphological appearance and which were homozygous for the chromosomes of the *pruinosa* interchanges. Therefore the usual method of using homozygous types had to be abandoned and all determinations made with crosses to strains that were only heterozygous for these interchanges. Fortunately the two types of offspring from each cross of the heterozygote to homozygous PT testers could be distinguished cytologically because of long familiarity with the sizes and other peculiarities of the chromosomes in the various

PT tester races. These incompatibilities in crosses probably are a consequence of structural changes in the chromosomes which cannot be detected by our method of examining the meiotic chromosomes at *MI* and possibly also of gene mutations that have occurred in the different species. There were fewer barriers between *pruinosa* and other species of the same section than between it and *stramonium* which belongs to a different section of the *Datura* genus.

A cross was made between the tester race of *D. leichhardtii* and that of *D. pruinosa*. The 24 chromosomes in the hybrid were arranged as a $\odot^b 4 + \odot 4 + 8$ bivalents at the first meiotic metaphase in *PMC*. Therefore the tester races of these two species differ by two separate interchanges each of which consists of an interchange between two non-homologous chromosomes. Eight of the chromosomes of *pruinosa* have the same end arrangements as eight of the chromosomes of the *leichhardtii* tester race.

This hybrid was back-crossed to the *leichhardtii* tester. Among the offspring, there were plants which also showed $\odot 4 + \odot 4$. This back-crossing and recovery of the two circles was repeated twice more. By this time the plants resembled *leichhardtii* in external morphology. Such a plant when selfed gave among its offspring some plants that showed only 12 bivalents at *MI*. A backcross to *leichhardtii* was needed to distinguish between those bivalent plants which were homozygous for the four *leichhardtii* chromosomes and those homozygous for the four *pruinosa* chromosomes which are involved in the two $\odot 4$ configurations. The former showed only bivalents in the backcross hybrid, the latter showed $\odot 4 + \odot 4$.

Two strains of *leichhardtii*, differing slightly in external appearance, but both homozygous for the end arrangements of these four *pruinosa* chromosomes, were obtained by this method. They were continued by selfing and were used in place of the tester race of *pruinosa* in making crosses. This method of backcrossing apparently removed some barriers to crossability so that viable hybrids were obtained with *D. inoxia* and *D. meteloides*. The hybrid with *inoxia* type 1 showed a $\odot 8 + 8$ bivalents; the hybrid with *inoxia* type 2 showed a $\odot 8 + \odot 4 + 6$ bivalents. The hybrid with the tester race of *meteloides* showed a $\odot 8 + \odot 4 + \odot 4 + 4$ bivalents. At this time also a cross with *leichhardtii* type 2 was made. The hybrid showed a $\odot 4 + 10$ bivalents. This single interchange indicates that the end arrangements of only two *leichhardtii* type 2 chromosomes are different from those of *pruinosa*.

No viable hybrid could be obtained with either of these extracted lines of *leichhardtii* when they were crossed to the tester race of *stramonium*. Fortunately we had previously developed strains of *leichhardtii* which included in their chromosome complement the end arrangements of the four *stramonium* chromosomes which are different from the tester race of

leichhardtii.^{4, 6} A successful cross could be made by using *in place* of the tester race of *stramonium* such a strain of *leichhardtii*. That is, the compatible cross was between two extracted lines of *leichhardtii*, one from a previous *leichhardtii-pruinosa* cross and the other from a previous *leichhardtii-stramonium* cross. This indirect approach, using *leichhardtii* as the bridging species, made possible a comparison of the end arrangements of the *pruinosa* chromosomes with those of our standard *PT1* of *stramonium*. The hybrid showed a $\odot 4 + \odot 4 + \odot 6 + 5$ bivalents. Usually the $\odot 6$ was broken leaving a chain of 6 instead of the $\odot 6$, as shown in figure 1. It was concluded that only five of the chromosomes of *pruinosa* have the same end arrangements as those of *PT1*. The other seven chromosomes are different. In terms of *PT1*, two chromosomes are involved in each of two interchanges and three in the third. In order to identify these seven chromosomes, a study was made of the chromosome arrangements in crosses to selected *PT*'s of *stramonium* and chromosomal types of other species.

This F_1 hybrid was backcrossed to another extracted line of *leichhardtii* carrying the *PT1* chromosomes⁷ with the object of introducing *stramonium* genes and consequently of building up strains more like *stramonium*. From the offspring a plant was selected which showed $\odot 4 + \odot 4 + \odot 6$. This also was backcrossed to an extracted line of *leichhardtii* which carried the same *PT1* chromosomes but which was nearer *stramonium* in appearance. Some of the offspring from this cross showed only one or two configurations. With them it was decided to study each of the interchanges separately and since these plants now showed more resemblance to *stramonium*, attempts were made to make crosses directly to *stramonium* *PT* testers.¹

That two of the *pruinosa* chromosomes are 1·18 and 2·17⁸ was proved in the following manner. From the self of a plant which showed a $\odot 4 + \odot 4$ there was obtained a plant which showed the same two circles of four. These were cytologically distinguishable. One circle seemed to consist of four *M*-sized chromosomes.^{1, 9} The other looked like the heterozygous *PT2* configuration (1·2-2·17-17·18-18·1) judging by the sizes of the chromosomes (*L*, *l*, *m*, *m*). That two of the chromosomes in

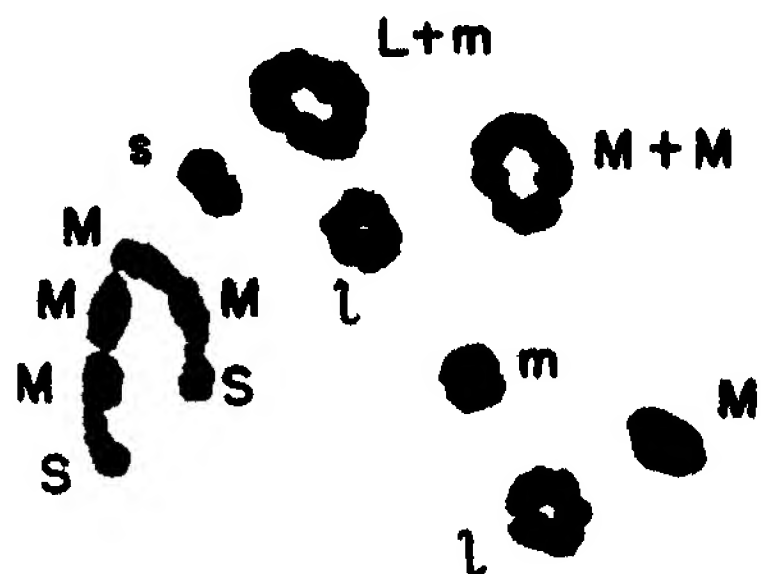


FIGURE 1

Arrangement of 24 chromosomes in extracted *pruinosa* \times extracted *stramonium* hybrid as $\odot 4 + \odot 4 +$ chain 6 + 5 bivalents. The size of each chromosome in the chain (broken circle) is given. Otherwise sizes are indicated for pairs of chromosomes in bivalents and in two circles.

the circle really were 1·2 and 17·18 was known because of previous backcrosses to *leichhardtii* strains homozygous for *PT1* chromosomes. Since the plant resembled *stramonium* in appearance, a direct cross to homozygous *PT2* of *stramonium* was made. Proof that the other two chromosomes in this circle were 1·18 and 2·17 was given when one of the offspring of this cross to homozygous *PT2* showed only bivalents. It may be diagrammed as follows:

$$\begin{array}{l} \odot 4 \text{ gamete } a (1 \cdot 2 + 17 \cdot 18) \times PT2 \text{ gamete } (1 \cdot 18 + 2 \cdot 17) = \odot 4 \\ \quad \quad \quad " \quad b (1 \cdot 18 + 2 \cdot 17) \times \quad \quad \quad " \quad \quad \quad " \quad \quad \quad = \text{bivalents.} \end{array}$$

Only because the $\odot 4$ plant was producing 1·18, 2·17 gametes could one find among the offspring of this cross to homozygous *PT2* a plant showing all bivalents. These two chromosomes must have come from *pruinosa*.

That two of the *pruinosa* chromosomes are 9·14 and 10·13 was proved in the following manner. From a preliminary cross between the F_1 hybrid and homozygous *PT7* (9·10²⁰, 19·20¹⁰) there was obtained a plant which showed a configuration of six as follows:

$$\begin{array}{ccccccc} x \cdot y \cdot y \cdot 10^{10-10} 20 \cdot 19 & & & & & & \\ | & & | & & | & & | \\ x \cdot 9 \cdot 9 \cdot 10^{20-20} 20 \cdot 19 & & & & & & \end{array}$$

This indicated that the 9·10 chromosome is involved in the *pruinosa* interchange as well as in *PT7*.¹⁰

A cross was then made between a plant which showed the circle of four *M*-sized chromosomes (not heterozygous for *PT2*) and homozygous *PT11* (11·13, 12·17, 14·18). This $\odot 4$ plant produced two kinds of viable gametes, those with *PT1* chromosomes (because of the previous backcrosses to *leichhardtii* strains homozygous for *PT1*) and those with *pruinosa* chromosomes. The *PT1* type of gamete crossed to *PT11* would give a $\odot 6$. If one of the chromosomes in the *pruinosa* interchange were the same as one in the *PT11* interchange, a $\odot 8$ would be expected. Actually among the offspring there was a plant which showed a $\odot 8$. Familiarity with the sizes and other morphological peculiarities of the chromosomes, especially the interchanged chromosomes of *PT11*, permitted the following representation of the $\odot 8$:

$$11 \cdot 12 - 12 \cdot 17 - 17 \cdot 18 - 18 \cdot 14 - 14 \cdot a - a \cdot b - b \cdot 13 - 13 \cdot 11$$

That the *a·b* chromosome is the 9·10 chromosome had been indicated by the cross to *PT7* and was verified by a cross to homozygous *PT23* (2·1·9, 10·). Among the offspring was a plant which showed a chain of 6 as follows:

$$1 \cdot 2 - 2 \cdot 1 \cdot 9 - 9 \cdot x - x \cdot y - y \cdot 10 - 10 \cdot$$

The \odot 8 obtained from the cross with *PT*11 indicated that one of the two *M*-sized chromosomes, 11·12 or 13·14, is involved in the *pruinosa* interchange. That this is not the 11·12 chromosome was learned from crosses with *PT*3 (11·21, 12·22) and *PT*34 (11·12²⁴, 23·24¹²). Therefore the chromosome represented as *x*·*y* in the offspring from crosses with *PT*7 and *PT*23 is the 13·14 chromosome.

In order to determine the end arrangements of these two *pruinosa* chromosomes, crosses were made with those *PT*'s in our collection which involve both the 9·10 and 13·14 chromosomes. From a cross with homozygous *PT*62 (9·14, 10·13) there was obtained a plant which showed only bivalents. Therefore the chromosomes in this *pruinosa* interchange are 9·14 and 10·13.

The three remaining unknown *pruinosa* chromosomes, which take part in the \odot 6, were determined to be 11·16, 12·22 and 15·21 in the following manner.

From a preliminary cross between the *F*₁ hybrid and homozygous *PT*4 (3·21, 4·22) there was obtained a plant which showed a \odot 8. This indicated that one of the chromosomes involved in the *PT*4 interchange is the same as one in the *pruinosa* interchange. From a study of size differences, it was surmised that this is the 21·22 chromosome of *PT*1.

A plant showing the \odot 6 was heterozygous for this *pruinosa* interchange and was expected to produce two kinds of viable gametes, those with *PT*1 chromosomes (because of the previous backcrosses to *leichhardtii* strains homozygous for *PT*1 chromosomes) and those with the three *pruinosa* chromosomes. This plant was crossed to a homozygous *PT*3 (11·21, 12·22) line extracted from the *quercifolia* tester race.^{8, 11} All offspring showed a \odot 4 but two kinds could be distinguished cytologically. The *SMMS* circle was heterozygous *PT*3 and was discarded. The other circle consisted of four *M*-sized chromosomes and was the product of the union of the three *pruinosa* chromosomes and the chromosomes from *PT*3. Since only four chromosomes were in the circle, two conclusions could be drawn: that both the 11·12 and 21·22 chromosomes of *PT*1 are involved in the *pruinosa* interchange and that one of the *PT*3 chromosomes was identical, in so far as end arrangements are concerned, with one of the *pruinosa* chromosomes (either 11·21 or 12·22). These two chromosomes had formed a bivalent. Examination of the bivalents showed no size inequalities between the two members of each bivalent.

A plant showing a \odot 6 was also crossed to homozygous *PT*91 (11·22, 12·21). The offspring that were merely heterozygous *PT*91 could be discarded because they showed only a \odot 4. Others showed a \odot 6. This \odot 6 indicated that, although both the 11·12 and 21·22 chromosomes of *PT*1 are involved in the *pruinosa* interchange, no *pruinosa* chromosome can be represented as either 11·22 or 12·21.

The tester race of *D. discolor*¹² has the following interchanged chromosomes: 1·11, 2·17, 12·22, 15·21, 16·18. A cross was made between this tester race and a plant having the ⊙ 6 (heterozygous *pruinosa* interchange). As mentioned above, this ⊙ 6 plant produced two kinds of viable gametes, those with *PT*1 chromosomes and those with *pruinosa* chromosomes. Offspring from the union of the former and *discolor* gave a ⊙ 10 and were discarded. Some offspring showed a ⊙ 6. They came from the union of a *discolor* gamete and the three *pruinosa* chromosomes. This meant that two of the *discolor* chromosomes have the same end arrangements as two of *pruinosa*. The 2·17 was ruled out because it was known to be in another interchange (previously described). The 1·11 and 16·18 chromosomes were ruled out because of the fact that the 1·18 chromosome also had been identified in the same interchange with 2·17. Therefore the two chromosomes had to be 12·22 and 15·21. This cross to *discolor* therefore showed that it is the 12·22 chromosome of *pruinosa* which is the same as one of the *PT*3 chromosomes, and *not* the 11·21 chromosome of *PT*3.

It was also noticed among the offspring of the *discolor* × *pruinosa* chromosome cross that one bivalent consisted of an unequal pair of chromosomes. In the cross to *PT*3 no unequal-sized pair had been seen. Therefore the 12·22 chromosome of *pruinosa* has the same length as the 12·22 chromosome of *PT*3. However, it is known that the 12·22 chromosome of *discolor*¹² is longer than the 12·22 chromosome of *PT*3. Therefore the unequal pair of chromosomes seen in the cross to *discolor* must be the (12·22)₂ pair.

If two of the *pruinosa* chromosomes are 12·22 and 15·21, then the third one must be 11·16. In order to verify this conclusion and to determine the sizes of the 11·16 and 15·21 chromosomes, a further cross was made to the tester race of *D. ferox*. The latter has the following interchanged chromosomes: 1·18, 2·17, 11·21, 12·22, 7·20¹⁶, 8·19, 15·16²⁰. Again a plant with a ⊙ 6 (heterozygous for the *pruinosa* interchange) was selected and crossed to *ferox*. Offspring from the union of the gamete carrying the *PT*1 chromosomes and *ferox* could be recognized cytologically and discarded. Other offspring showed a ⊙ 4 (heterozygous *PT*2) plus a configuration of eight chromosomes. The latter was either a ⊙ 8 or a ⊙ 4 to which a chain of 4 was attached. The latter was examined carefully because it furnished the needed proof. It may be diagrammed as follows:

$$\begin{array}{ccccccc}
 19 \cdot 8-8 \cdot 7-7 \cdot 20^{16-16} & 16 \cdot 11-11 \cdot 21 & & & & & \\
 | & | & & | & & & \\
 19 \cdot 20^{20-20} & 16 \cdot 15-15 \cdot 21 & & & & &
 \end{array}$$

The junction of the ·16 and ·20 ends is marked by the intersection of the

circle and chain. There were four *M*-sized chromosomes in the circle. The 11·16 chromosome is slightly larger than the 15·21 chromosome.

In table 1 are shown the chromosomes of *D. pruinosa* in terms of *PT*1 of *stramonium*. For comparison, the latter are also shown as well as the chromosomes of the tester race of *D. leichhardtii*.

The number of interchanges by which one species differs from another depends in part upon what race is chosen as the tester race for each species, and consequently cannot be used as sole criterion for determining their phylogenetic relationship. However, similarity of certain arrangements of chromosome ends is very suggestive. The 11·16 chromosome which is singularly absent from all races and species of the *Stramonium* section which we have in our collection, occurs in both *pruinosa* and *leichhardtii* which belong to another section of the genus *Datura*.

TABLE 1
CHROMOSOMES OF THE TESTER RACES OF *Stramonium*, *Pruinosa* AND *Leichhardtii*

<i>Stramonium PT</i> 1	<i>Pruinosa</i>	<i>Leichhardtii</i>
1·2	1·18	1·18
3·4	3·4	3·4
5·6	5·6	5·6
7·8	7·8	7·8
9·10	9·14	9·10
11·12	11·16	11·16
13·14	13·10	13·14
15·16	15·21	15·12
17·18	17·2	17·2
19·20	19·20	19·20
21·22	12·22	21·22
23·24	23·24	23·24

¹ Bergner, A. D., Satina, S., and Blakeslee, A. F., these PROCEEDINGS, 19, 103-115 (1933).

² Safford, W. E., *Jour. Wash. Acad. Sci.*, 11, 173-189 (1921).

³ Blakeslee, A. F., *Univ. Pennsylvania Bicentennial Conf.* In Cytology, genetics, and evolution, 37-46 (1941).

⁴ Blakeslee, A. F., *Carnegie Inst. Wash. Year Book*, 36, 38-39 (1937).

⁵ ⊙ signifies "circle of."

⁶ Blakeslee, A. F., *Ibid.*, 40, 220-224 (1941).

⁷ For the sake of brevity "chromosomal end arrangement" will not be repeated but is to be understood whenever the word chromosome is used.

⁸ Interchanged chromosomes are in boldface type.

⁹ Satina, S., Bergner, A. D., and Blakeslee, A. F., *Amer. Jour. Bot.*, 28, 383-390 (1941).

¹⁰ Where the interchanged segments are limited to the terminal regions of chromosomes, these regions are represented by raised numerals. In satellite chromosomes only the satellites may be involved. This kind of interchange when heterozygous induces the "necktie" type of configuration.

¹¹ Bergner, A. D., and Blakeslee, A. F., these PROCEEDINGS, 18, 151-159 (1932).

¹² Bergner, A. D., and Blakeslee, A. F., *Ibid.*, 21, 369-374 (1935).

A COMPLEX INDUCED REARRANGEMENT OF *DROSOPHILA*
CHROMOSOMES AND ITS BEARING ON THE PROBLEM OF
CHROMOSOME RECOMBINATION

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Among the F_1 larval progeny of an irradiated *Drosophila melanogaster* male of the Oregon-R stock and an untreated Oregon-R female, there appeared one individual whose salivary-gland chromosomes revealed a complex rearrangement involving at least 32 breaks. The positions of 30 points of breakage were determined; the others remain of uncertain location. Treatment of the father included exposure to 4000 roentgens of x-rays, followed by near infrared radiation for a period of 144 hours. Details of these experiments will be furnished in another publication; for the present it seems adequate to note that the induction of the breaks is in all probability attributable to the x-rays, since no chromosome rearrangement has been found among a long series of controls derived from fathers exposed only to the near infrared.

The breakage points, recorded below, that were utilized in this complex rearrangement have been determined within the limits of accuracy imposed by the few nuclei available for study. They are:

1B, 3C, 5D, 11F, 13E, 15F in the X-chromosome;
24D, 33E, 40, 41, 42C, 49F, 51E, 56B in the second chromosome;
64C, 70C, 76A, 84F, 85F, 86E, 87B, 91A, 93F, 96B, 96E, 98A, 98E, 99D,
99F in the third chromosome;
102 in the fourth chromosome.

Identification of the lettered subdivisions was made from the maps drawn by C. B. Bridges and P. N. Bridges.

The complete pattern of recombination could not be determined cytologically because of the confusion of pairing in the chromocentral regions. However, the following sequences have been inferred from observations of continuity between the various displaced sections. (Points of recombination are indicated by sloping lines (/); the centromere is indicated by the symbol cm., a chromocentral region by chr.) Nine separate sequences were identified, and are listed below:

(a) Tip 3R-99F/99D-98E/84F-85F/99F-99D/98A-96E/96B-93F/40-33-
E/70C-64C/56B-51E/87B-91A/15F-cm. of X

- (b) Tip of $X-1B/41$ to cm. of $2R$
- (c) Tip of 4 to $102/86E-87B/76A-80?$
- (d) chr. of $?/91A-93F/40$ -cm. of $2L$
- (e) Tip of $2R-56B/42C-49F$ /chr. of $?$
- (f) chr. of $?/11F-13E/98E-98A/84F-81?$
- (g) Tip of $3L-64C/76A-70C/49F-51E$ /chr. of $?$
- (h) Tip of $2L-24D/3C-1B/5D-11F/15F-13E$ /chr. of $?$
- (i) chr. of $?/41-42C/33E-24D/3C-5D/85F-86E/102$ -cm. of 4

These sections include all of the essentially euchromatic portions of the chromosomes, except for the section $96B$ to $96E$, which was not detected and which may be assumed to be missing. Only two of the breaks in the proximal heterochromatic regions could be determined with any degree of certainty; these were in 40 of $2L$ and in 41 of $2R$. The parts separated by these two breaks maintained the constant pattern of recombination that is indicated above. In addition, at least two other breaks must have been produced in order to provide a complete set of chromosomes, each with a centromere, capable of surviving the many divisions leading to the formation of the fully developed larva. Such a viable complement could be realized if breaks had occurred also in divisions 80 and 81 to give the following sequences:

- Tip of $3R$ to cm. of X (as detailed in (a) above)
- Tip X to cm. $2R$ (as in (b) above)
- Tip $4-102/86E-87B/76A-80/91A-93F/40$ -cm. $2L$
- Tip $2R-56B/42C-49F/81-84F/98A-98E/13E-11F/80$ -cm. $3L$
- Tip $3L-64C/76A-70C/49F-51E/81$ -cm. $3R$
- Tip $2L-24D/3C-1B/5D-11F/15F-13E/41-42C/33E-24D/3C-5D/85F-86E/102$ -cm. 4

On this interpretation the entire complex can be resolved into seven independent exchanges or groups of exchanges: four of them each involving two breakage points, one with four, and two others with ten points each. They are the following: $93F/40$; $96B/96E$ (deficiency); $24D/3C$; $102/86E$; $15F/91A/80/11F$; $98A/99D/99F/85F/5D/1B/41/13E/98E/84F$; $87B/51E/81/49F/70C/33E/42C/56B/64C/76A$.

Although this represents the simplest pattern of recombination, it does not appear on purely cytological grounds to be the most probable one, for the reason that the assumed sequences $80/91A$, $80/11F$ and $81/51E$, $81/49F$ have not been observed, as is usually possible in such cases (for example, the sequences related to the 40 and 41 breaks in the second chromosome). There are also some indications that certain of the ends bordering on the chromocenter, as those of sequences f and g , are associated with material of

the nucleolus, and this suggests that the nucleolus-organizing region may have been transferred from the X-chromosome to the chromocenter of another chromosome. Under such conditions, the number of breaks involved would be increased to at least 34, of which as many as 26 might contribute to the formation of a single rearrangement, the remaining 8 pairing in twos. Whatever the actual pattern of recombination may be, the complexity of the rearrangement in itself merits consideration because of the light it sheds on problems of chromosome breakage and recombination.

Discussion.—The most extensive derangement of *Drosophila* chromosomes previously observed in this laboratory involved 14 breaks. One reason why complex, multiple-break recombinations are encountered much less frequently than the simpler types is that zygotes receiving such altered chromosomes depend for their survival on the chance union of several broken ends to form viable combinations, while the zygotes receiving acentric and dicentric chromosomes perish in the early divisions to provide the dominant lethals represented by unhatched eggs. Calculations made for the less complex rearrangements^{1, 2} indicate that with an increasing number of breaks the percentage of viable combinations decreases. Discovery of the rearrangement reported in the present paper has emphasized the need for determining the probability of such a viable combination. Calculations made to this end³ indicate that the chances for a balanced, viable reassociation of the segments separated by 32 breaks distributed among all the chromosomes are about one in thirty or forty. This frequency is high enough so that in itself it does not account for the failure to detect such changes in the ordinary quantitative experiment involving cytological inspection of several hundred pairs of glands. The fact that no other alterations of equal or greater complexity have come to light indicates that their appearance does not depend solely on chance recombination of fragments, but probably on factors limiting the production of rearrangements involving as many as 32 breaks. One such limiting factor is the number of regions of potential breakage immediately produced by the radiation; another is the chance that some potential breaks may undergo restitution. These questions have been discussed in a separate paper.⁴

Presumably, the points of potential breakage induced by the radiation that penetrates the nucleus are scattered at random along the chromosomes. Break frequency as revealed in the salivary-gland chromosomes has been found to be essentially at random; the breaks considered as a whole are distributed among the chromosomes in proportion to their lengths.^{1, 2} Since these findings are based on the analysis of a multitude of rearrangements, mostly with two breaks, the question remains whether the greater complexity of certain alignments might not be related to the accumulation of breaks within individual chromosomes or chromosome limbs. In the

rearrangement here reported, a large proportion of the breaks was found in the right limb of the third chromosome. Considering only the 27 breaks in the essentially euchromatic regions of the longer chromosomes (excluding the proximal heterochromatic zones), the following distribution was found: 6 in the *X*, 2 in 2*L*, 4 in 2*R*, 3 in 3*L*, 12 in 3*R*. If random distribution of ionization and of regions of potential breakage is assumed, as seems entirely justifiable from available physical evidence, and if recombination were at random, the observed breaks should also be at random. However, the probability of securing the observed distribution lies between 0.02 and 0.05, using the χ^2 test as a measure of significance. On the basis of this low probability, the distribution of breaks in this highly complex case might be referable to some factors interfering with randomness of recombination.

As has been pointed out by Bauer, Demerec and Kaufmann,¹ break distribution among the chromosome limbs does not agree with expectation even for the simpler cases. In the two-break cases, a preponderance of inversions was found as compared with translocations, and this has been attributed, in general, to spatial relationships favoring recombination between adjacent regions. Likewise, among the three-break cases there was an excess over expectation of rearrangements with all the breaks in one limb (3), and a dearth of those with each break in a different limb (1 : 1 : 1). Bauer² reports, however, that random distribution obtains in the four-break cases. Several complications preclude the application of reliable statistical tests to the high-break cases; they are few in number, and the proportion of expected viables in each group varies according to the number of chromosome limbs involved. Among slides examined by the writer there are 174 multiple-break cases distributed as follows: 98 with four breaks, 34 with 5 breaks, 22 with 6, 7 with 7, 7 with 8, 4 with 9, and 2 with 12 (breaks in the fourth chromosome are not included). Although this material does not lend itself to critical evaluation for the reasons given above, distribution of breaks in the 5- and 6-break cases does not appear to deviate widely from randomness. Casual inspection of the more complex rearrangements indicates a clumping of breaks; for example, one of the twelve-break cases had 8 breaks in one chromosome limb, and two in each of two others (8 : 2 : 2). The other had a 6 : 4 : 2 distribution. It should also be pointed out that, of the 76 rearrangements with five or more breaks, only two involve all five of the chromosome limbs. The overall impression gained from such a survey, although not conclusive, is that the breakage points detected in individual rearrangements (whether simple or complex) are not distributed at random among the chromosomes. In the light of this conclusion, some further consideration seems warranted concerning the mechanism whereby chromosomal rearrangements are produced.

Summary.—Among the larval progeny of an irradiated *Drosophila melanogaster* male there appeared one female whose salivary-gland chromosomes showed extensive rearrangement that involved at least 32 points of breakage, and probably some others. The complexity is worthy of note, since no rearrangement involving more than 14 breaks had previously been discovered in this laboratory. The breaks in this complex rearrangement are not distributed at random, but are aggregated particularly in the right limb of the third chromosome. Such non-random distribution, coupled with the fact of the occurrence of such a complex rearrangement, prompts further consideration of the factors involved in chromosome recombination.

¹ Bauer, H., Demerec, M., and Kaufmann, B. P., *Genetics*, 23, 610–630 (1938).

² Bauer, H., *Chromosoma*, 1, 343–390 (1939).

³ Fano, U., *Proc. Nat. Acad. Sci.*, 29, 12–18 (1943). (Calculations will be found in the Appendix.)

MECHANISM OF INDUCTION OF GROSS CHROMOSOMAL REARRANGEMENTS IN DROSOPHILA SPERMS

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It has become progressively clear during recent years that the action of ionizing radiations on *Drosophila* sperm chromosomes develops through two fairly distinct stages, namely, "single-atom effects" and "combination effects."¹ Single-atom effects are individual alterations of the chromosomes, resulting in actual or potential breaks; and each of them arises from the activation (excitation or ionization) of a single atom during the treatment. According to physical information, atomic activations are produced at random and independently of each other throughout the chromosomal material and throughout the duration of the irradiation. The products of single-atom effects remain separate from one another during the spermatozoon stage. Combination effects occur after fertilization, when the previously produced single-atom effects become apparent, so that the broken ends derived at the different points of rupture can be shuffled and rejoined in new combinations.

It is generally recognized that the laws governing the single-atom effects are known in their essentials, although the corresponding mechanism of action is not. The laws governing the combination effects are less well known. The simplest hypothesis is that, following fertilization, broken

chromosome ends may join new partners under conditions of free competition. Experimental evidence does not agree with the implications of this hypothesis;^{1, 2, 3} therefore it was found necessary to consider mechanisms of "restricted competition." Restrictions on the freedom of competition were thought to arise from spatial and structural relationships between different breaks, but could not be formulated in simple, unequivocal rules. Analogous factors appear to affect the occurrence of chromosomal rearrangements in *Tradescantia* microspores, where it also appears that chromosomal mechanical stresses may affect differentially the chance for any individual break to rejoin.⁴ All earlier discussions, whether based on the theory of free or of restricted competition, seem to have carried in the background one unformulated assumption, namely, that the rejoining of one or more pairs of broken ends during the process of recombination does not affect the fate of other broken ends otherwise than by reducing the number of broken ends available for further recombinations. This would mean that the fate of one broken end does not influence actively the subsequent behavior of other broken ends.

In fact, the exclusion of any such active influence is not required by logical argument nor by experimental evidence. Stresses along chromosomes and interchromosomal pulls due to interlocking, for instance, may provide mechanisms by which the joining of two broken ends may actively influence the recombination of other broken ends. Therefore, the earlier "unformulated assumption" may serve as a tentative hypothesis only so long as it is not disproved by further experimental evidence. It has already been pointed out³ that the relative frequency of simple and complex rearrangements cannot easily be explained on the basis of existing ideas concerning the combination effects. The recent discovery⁵ of a complex rearrangement involving at least 32 breaks prompts us now to re-examine the question.

As far as the production of two-break rearrangements is concerned, the present interpretation seems to be satisfactory. The purpose of this paper is to determine how this interpretation should be extended in order to account for the available evidence on complex rearrangements. Two generally recognized results will be taken as a basis: (a) The primary action of x-rays consists of single-atom effects; that is, of individual breaks, produced independently of each other. The average total number of breaks is proportional to the radiation dosage, and the number within each sperm is governed by a Poisson distribution. (b) The observed frequency of two-break rearrangements is proportional to the square of the dosage. (The latter result holds when the dosage is sufficiently low; that is, when the frequency of all rearrangements is small as compared to one.) Evidence that this situation occurs in *Drosophila* has repeatedly been re-

viewed,¹ but the most direct evidence on the laws governing the production of individual breaks has been gathered in grasshopper material.⁶

Simple mathematical considerations, which are omitted for the sake of brevity, show that the following can be inferred from (a) and (b) within their limits of validity. The chance that any break produced within a sperm will become involved in a two-break rearrangement is proportional to the average number of other breaks present in the same sperm. The establishment of this inference represents a positive achievement, but it does not yet yield a sufficiently complete picture of the process of rearrangement. In fact, this picture might be filled in equally well in either of two alternate ways (as well as intermediate ones), namely: (1) Less than two breaks per sperm are frequently produced, in the usual range of dosage, but it is very likely that a two-break rearrangement will occur whenever two breaks happen to be available in the same sperm. (2) On the average, there are many breaks in every sperm, but each break has only a slight chance of combining with any other single break to give rise to a rearrangement. (In this case it must be assumed that most of the breaks "reheal," because otherwise unhealed breaks would cause dominant lethal effects to a much greater extent than has been observed.⁷)

To discuss the relationships between two-break and many-break rearrangements, it is well to consider (1) and (2) separately. On the basis of (1), it is possible to estimate from the observed frequency of two-break rearrangements that the total rate of production of breaks should be of the order of 0.4 per sperm per 1000 r x-rays. It is easily seen that this rate is grossly inadequate not only to account for even the occasional occurrence of a 32-break rearrangement within all the x-rayed material ever observed, but also to account for the observed rate of production of less complex rearrangements (e.g., of the 6-break ones). On the basis of (2), the inference established for two-break rearrangements at low dosage appears to represent a principle of "non-interference" between different breaks: the chance for one break to recombine with any other break is not affected by the presence of still other breaks in the same sperm; hence the total chance of recombination of one break is the sum of the chances of its recombining with each of the other breaks in the sperm. This principle of non-interference, which is founded upon experimental evidence as far as two-break rearrangements are concerned, is clearly related to the generally accepted "unformulated assumption" that was discussed above. That this principle applies to complex rearrangements as well may seem plausible; but it remains only a working hypothesis, to be tested by comparison with experimental evidence.

It is not easy to perform this test, because there is no single way in which to develop a mathematical theory from the working hypothesis and thus to obtain theoretical formulae for comparison with quantitative experimental

results. Several fragmentary considerations have been taken into account, however, that permit us to state with some assurance that the working hypothesis should be rejected. One such consideration runs as follows: In order that very complex rearrangements may occur even occasionally, the rate of occurrence of breaks should be much larger than previously suspected; and conversely, the chance for any pair of breaks in the same sperm to give rise to a two-break rearrangement should be very small, about $1/100$ or even less. Under these conditions and under the assumption of non-interference between different breaks, complex rearrangements should consist almost exclusively of superimposed two-break rearrangements; while the frequency of more complex "contacts" should be negligible. No such extreme effect has been observed.⁸ (It is worth noting, however, that more "2 + 2" rearrangements have actually been found than was to be expected on certain other bases.⁸)

To account for the experimental evidence on the frequency and type of complex rearrangements, therefore, it seems necessary to assume the existence of an active influence between different breaks. Although the chance for any one break to become involved in a rearrangement is to begin with simply proportional to the average number of other breaks in the same sperm, this chance should become materially greater when other breaks themselves become involved in a rearrangement. The assumption of combination effects of this sort does not seem unreasonable, in view of the situation prevailing when the sperm opens up after fertilization. The establishment of an illegitimate union between different parts of the chromosomal complex may well perturb the mechanical phenomena that are developing, so as to draw into rearrangement "potential" breaks (i.e., single-atom effects) that would have rehealed otherwise. On this basis the non-random concentration of breaks observed in parts of the chromosomal complex⁵ could be easily understood, since mechanical perturbations cannot be expected to affect the whole complex uniformly.

Although our assumption may seem fairly satisfactory, it must be stated that the requirements set by the evidence on complex rearrangements are not quite specific, implying only that chromosomal breaks become more readily available to take part in a rearrangement when the rearrangement itself begins to develop from other breaks. Breaks that become more readily available do not logically need to be drawn from the same source (single-atom effects) as the breaks that started the rearrangement. The alternate hypothesis, that breaks are produced by combination effects in some other way does not seem very reasonable in view of general evidence on the behavior of chromosomes; and it would not even be mentioned here, except that some evidence in its favor, even though weak, does exist. The arguments considered earlier in this paper have referred to evidence on the total number of complex rearrangements observed per two-break rearrange-

ment, that is, on the relative frequency of complex and simple rearrangements in general. Data are also available, however, on the variation of this relative frequency with x-ray dosage throughout the experimental range (that is, between 1000 and 5000 r); this variation is found to be small.^{3, 8} This further result cannot be easily explained on the assumption that all breaks originate from single-atom effects. Even though single-atom effects are assumed to be very numerous in each sperm at 1000 r, they should be five times as numerous at 5000 r. The number of breaks constituting raw material on which combination effects can draw during the production of complex rearrangements should thus increase substantially with increasing dosage. This would in turn bring about a corresponding increase in the number of breaks observed per rearrangement, that is, an increase in the "average complexity" of rearrangements. A very small variation in the average complexity is, on the contrary, the result anticipated if it is assumed that the supply of breaks for complex rearrangements does not increase proportionally to the dosage, but much more slowly than that, if at all. This assumption would not necessarily mean that the existence of available breaks is wholly unrelated to the radiation treatment, but that it is related to it through some other mechanism than the single-atom effects.

The fact that the average complexity of x-ray-induced rearrangements depends but little on the dosage might occasion some speculation as to the average complexity of spontaneous rearrangements, inasmuch as these rearrangements might be started by single-atom effects that are analogous to, though much less frequent than, those induced by radiation. Very little can be stated on this subject, by reason of the scarcity of experimental data, except that, in proportion to two-break rearrangements, there are certainly more complex ones in x-ray-treated than in untreated material. For instance, there was found, on the average, in treated material one rearrangement among the types classified as "3-" or "4-" or "2 + 2-" cases per every 2.15 two-break rearrangements. Although this ratio does not vary significantly throughout the dosage range from 1000 to 5000 r,^{3, 8} none of the spontaneous rearrangements (numbering 50 to 100) ever detected within all species of *Drosophila* appears to involve more than two breaks. However, the chance of observing occasional more complex rearrangements in nature might have been adversely affected by selection.

Summary.—Evidence on the occurrence of radiation-induced chromosomal rearrangements indicates that breaks become more readily available to take part in a rearrangement after the rearrangement begins to develop from other breaks. The simplest possible mechanism that can be postulated is that a large number of potential breaks is produced in each sperm by the usual x-ray treatment, and that each break has a chance of rehealing that is originally large but can be substantially lessened by some mechanical

perturbation arising after fertilization when a rearrangement happens to be started by two other breaks. Consideration of further experimental evidence may indicate, however, that some of the breaks involved in complex rearrangements may not have been produced initially by radiation.

Appendix.—To evaluate the chance of survival of organisms carrying complex chromosomal rearrangements, the following mathematical problems have been considered. Let l chromosome limbs be broken at b points: (1) in how many different ways can the resulting broken ends rejoin regardless of whether the final configuration contains polycentric or acentric sections? (2) In how many different ways can the same broken ends rejoin under such conditions that the final configuration contains no polycentric or acentric section? The answer to the first question is known to be:⁸

$$1 \times 3 \times 5 \times \dots \times (2b - 1) = \frac{(2b)!}{b! 2^b} \quad (1)$$

The answer to the second question is:

$$(b - 1)! 2^{b-l} \quad (2)$$

Assuming that all recombinations arising from b breaks in l limbs are equally probable, the average chance of survival of such a recombination is given by the ratio of (2) to (1), that is:

$$\frac{lb! (b - 1)! 2^{b-l}}{(2b)!} = \frac{l}{b2^l} \left/ \binom{2b}{b} \right/ \left(\frac{1}{2} \right)^{2b} \quad (3)$$

Using Stirling's formula, when $b \gg 1$, this formula becomes approximately:

$$\frac{l}{2^l} \sqrt{\frac{\pi}{b}} \quad (3')$$

For example, when $b = 32$, $l = 6$, the quantity (3') is 0.03. In other words, about 3 per cent of recombinations involving 32 breaks distributed among all chromosomes of *D. melanogaster* should survive.

This calculation does not apply straightforwardly to the evaluation of the probability of observing a rearrangement such as that described by Kaufmann,⁶ for several reasons. First, it is not true that all recombinations arising from b breaks in l limbs are equally probable, because the actual distribution of breaks into "contacts" is known to affect the probability of recombination. One should therefore calculate the numbers of viable and unviable recombinations for the special class of rearrangement considered; for instance, for the "10 + 10 + 4 + 2 + 2 + 2" rearrangements. General formulae for this purpose are not available. Second, the calculation per-

formed above does not exclude the possibility that some broken ends may rejoin in the original order. Finally, recombinations involving acentric sections (deficiencies) may occasionally survive when these sections are sufficiently short; this actually happened in the case described by Kaufmann.⁸

We think, however, that the results obtained from (3) or (3') would not be greatly affected even if all objections could be taken into account quantitatively, and that therefore these formulae may offer a fair indication of the chance of survival of complex rearrangements.

¹ Cf. Muller, H. J., *Cold Spring Harbor Symposia*, 9, 151-167 (1941).

² Cf. Kaufmann, B. P., *Ibid.*, 9, 82-92 (1941).

³ Cf. Fano, U., *Ibid.*, 9, 113-120 (1941).

⁴ Sax, K., *Proc. Nat. Acad. Sci.*, 28, 229-233 (1942).

⁵ Kaufmann, B. P., *Ibid.*, 29, 8-12 (1943).

⁶ Carlson, J. G., *Ibid.*, 27, 42-47 (1941).

⁷ Fano, U., and Demerec, M., *Genetics*, 26, 151 (1941). Pontecorvo, G., *Jour. Genetics*, 43, 295-300 (1942).

⁸ Bauer, H., Demerec, M., and Kaufmann, B. P., *Genetics*, 23, 610-630 (1938). Bauer, H., *Chromosoma*, 1, 343-390 (1939).

THE EFFECT OF CENTRIFUGING UPON THE PRODUCTION OF X-RAY INDUCED CHROMOSOMAL ABERRATIONS

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Previous investigations¹ have shown that differential sensitivity to x-rays of *Tradescantia* microspores seems to be related to factors which change the spatial and structural relations of the chromosomes and alter their capacity for movement. If such mechanical factors are involved, any treatment which would tend to increase the stress or movement of the chromosomes during or immediately following irradiation should increase the incidence of chromosomal aberrations. This hypothesis has been tested by subjecting *Tradescantia* microspores to centrifugal forces during and following x-ray treatment.

The types of aberrations produced by x-rays and the relation between dosage and aberration frequency have been described in an earlier paper.² Irradiation at prophase produces chormatid breaks in which one or both sister chromatids may be affected. The aberrations are of two general types: simple deletions or 1-hit aberrations, and fusion or exchanges be-

tween chromatids of different chromosomes, and since these involve two breaks they are classed as 2-hit aberrations. When the cells were irradiated during the resting stage the aberrations observed at the following metaphase include dicentric and ring chromosomes, minute deletions, and occasional simple terminal deletions. Of these only the rings and dicentrics have been included in the present investigation. Prophase stages are much more sensitive than is the resting stage as measured by the incidence of chromosomal aberrations.

An International clinical centrifuge was used for most of the experiments and was operated at 2080 r. p. m. *Tradescantia* inflorescences were wrapped in moist paper and placed at the bottom of the cups. Irradiation while centrifuging the buds presented some difficulty. The aluminum cover and bronze cups of the centrifuge reduced the radiation intensity to 6 roentgens per minute. The dosage could not be measured directly and had to be estimated by the time of exposure, but the relative doses for the centrifuged and control series were checked by placing the dosimeter on the floor to one side of the centrifuge. Dosimeter readings were obtained for exposures made before centrifuging. The dosimeter, built by Dr. Cloud of M. I. T., was designed so that the dosage in roentgen units was registered in the control room.

When raying and centrifuging simultaneously the centrifuge was run continuously in some experiments and in others it was turned on and off alternately every half minute. During the off periods the revolution was sufficient to keep the cups horizontal, although a difference of one or two centimeters would have had little effect on the dosage, since the distance from the x-ray tube to the centrifuge was about 50 cm. In the control experiments the inflorescences were placed in the cups and rayed without centrifuging, but with the cups at the operating level, or the centrifuge was operated just fast enough to keep the cups horizontal—about 400 r. p. m.

When centrifuging followed irradiation, the inflorescences were rayed at 70 roentgens per minute. Centrifuging was started about five minutes after raying and continued for 30 minutes. Both intermittent and continuous centrifuging were used.

A control experiment showed that centrifuging alone had no effect on the frequency of chromosomal aberrations. Microspores which had been centrifuged for 20 minutes at prophase showed only 0.1 per cent of chromatid aberrations, which is approximately the normal frequency for untreated *Tradescantia* microspores.

The effects of centrifuging *Tradescantia* microspores during or after irradiation are shown in table 1. The data presented in part A of the table are combined from eight different experiments, covering a period of several months, and involving the analysis of 63,238 chromosomes. Resting cells

which were irradiated while being centrifuged have twice as many aberrations as those which were irradiated only. Irradiation of resting cells followed by centrifuging was no more effective than irradiation alone. There was no effect of either continuous or alternating centrifuging following irradiation.

The effect of centrifuging at the time of irradiation is shown for cells treated at prophase in part *B* of table 1. These data are derived from five different experiments involving 28,110 chromosomes. There is a very definite and significant increase of chromatid aberrations when the cells are centrifuged during irradiation, but no significant increase when the cells were centrifuged following irradiation.

Chromosomal aberration frequencies are increased both in the resting stage and at prophase when irradiation is accompanied by centrifuging the microspores. Centrifuging following irradiation has little or no effect on the incidence of either chromosome or chromatid aberrations. Centrifuging following irradiation might be expected to increase aberration frequency since fractional dosage experiments and the effect of varying x-ray intensity have shown that the production of aberrations may continue for 20–40 minutes after irradiation. There is some increase in chromatid aberrations resulting from centrifuging after raying, but the differences are not statistically significant. The absence of a significant effect when centrifuging follows irradiation may be due in part to the delay of about five minutes in shifting the material to the centrifuge, and in part to a rapid decline of potential fusions after irradiation. Although potential fusions persist in some cases for 20 to 40 minutes, in some experiments all fusions were completed in less than 20 minutes after the cessation of raying.

It has long been known that various cell constituents can be rearranged by centrifuging. Usually the nuclei occupy centrifugal positions, and occasionally nucleoli can be separated from the nucleus. More recently Kostoff³ found that the chromosomes of a cell can be shifted by centrifugal forces and in some species the chromosomes may be broken by centrifuging at 2500–3500 r. p. m. Even in the resting stage it is probable that centrifugal forces would cause some stress on the chromosomes, either indirectly by the movement of the denser nucleoli, or directly by differential movement of the chromonemata due to spatial relations. At prophase there should be considerable opportunity for induced chromosome movement since at this stage the chromosomes are partially contracted and do not completely fill the nucleus.

Any stress imposed upon the chromosomes during irradiation would be expected to increase the frequency of chromosomal aberrations. Most of the breaks produced by x-rays undergo restitution and no permanent effect is produced. Only when broken ends of chromosomes get out of alignment do illegitimate fusions occur which result in chromatid and chromo-

some aberrations. Stresses induced in the chromosomes at the time of breakage would separate the broken ends and promote illegitimate associations. Presumably this is the mechanism involved in the centrifuging of cells during irradiation.

Summary—*Tradescantia* microspores which were centrifuged during irradiation with x-rays were found to have a higher frequency of chromosomal aberrations than microspores which were irradiated only. The effect of centrifuging is attributed to the stresses imposed upon the chromosomes which cause the broken ends to separate and facilitate illegitimate unions of broken chromosomes. Thus chromosome movement appears to be an important secondary factor in the production of chromosomal aberrations by x-rays.

TABLE 1
EFFECT OF CENTRIFUGING X-RAYED MICROSPORES
A. Rayed during resting stage. 63,238 chromosomes

DOSE R. U.	METHOD USED*	CONTROL	PER CENT CHROMOSOME BREAKS CENTRIFUGED	DIFF.
≈ 120 r.	C, D	2.2	4.4	2.2 ± 0.24
320 r.	C, F	15.1	16.8	0.5 ± 0.9
320 r.	A, F	16.5	15.9	

B. Rayed at prophase. 130-160 r. 28,110 chromosomes

METHOD USED*	PER CENT CHROMATID BREAKS					
	1-HIT		2-HIT		DIFF.	2-HIT
	CONT.	CENT.	CONT.	CENT.		
A + C, D	4.6	7.6	8.9	12.1	3.0 ± 0.4	3.2 ± 0.9
A, F	5.4	6.6	6.7	8.0	1.2 ± 1.0	1.3 ± 1.4

* C—continuous centrifuging. A—alternating centrifuging. D—centrifuged during irradiation. F—centrifuged following irradiation.

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¹ Sax, K., and Swanson, C. P., *Amer. Jour. Bot.*, 28, 52 (1941).

² Sax, K., *Symposia on Quant. Biol.*, 9, 93 (1941).

³ Kostoff, D., *Cytologia*, 8, 420 (1938).

INCREASE IN MAMMARY CARCINOMA INCIDENCE FOLLOWING INOCULATIONS OF WHOLE BLOOD*

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This is a report of an investigation of the possible rôle of blood in influencing the incidence of mammary carcinoma in mice. It is part of some work started in 1941 to try to open up new leads in and to further the study of a non-mendelian (though transferable from generation to generation maternally) influence affecting the incidence of mammary carcinoma.

In this particular experiment we have been able to conclude observations on the first group of mice. The last animals have just died at 736 days of age. Since the earlier tabulations the results have become increasingly significant. The probability that the results are due to chance alone shows odds of 1 to 10,390, that a random sample would give as great or greater deviation.

The recipient and control mice were of the second inbred generation following the foster nursing of inbred "high tumor" Jackson Laboratory C3H mice on "low tumor" inbred C57 black mice.

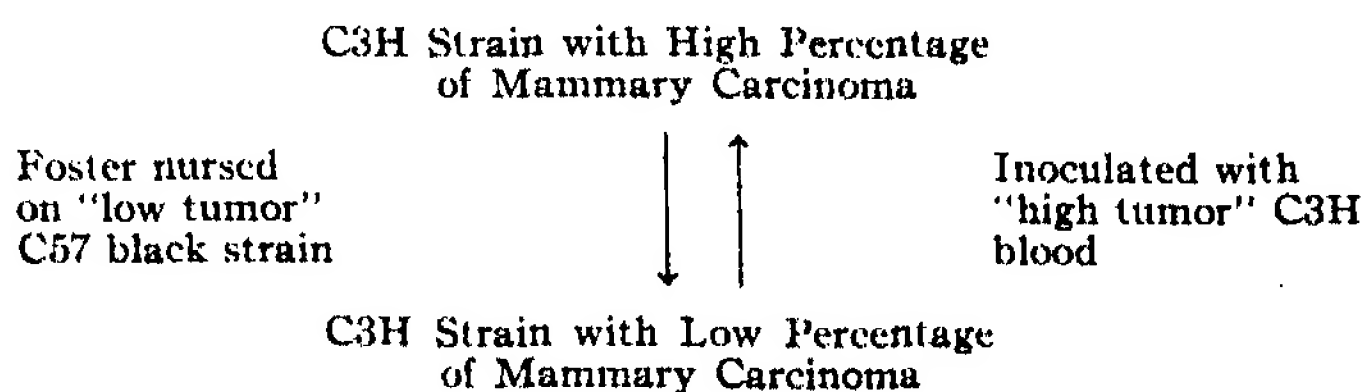


FIGURE 1

Plan of experiment showing method of securing "low tumor" C3H strain and the set-up for attempting to return it to "high tumor" strain.

At weaning time a male and four litter-mate females were placed in each compartment of a mouse box. Breeding was allowed to proceed normally and the young of each litter were removed by the time they were four weeks of age. Two females of each group of four litter-mates were injected subcutaneously with 0.5 cc of whole blood diluted with an equal part of distilled water. The recipients were 1 to 3 months of age. Normal males and females of the Jackson Laboratory C3H high tumor strain were used as donors. Most of the donors were young, non-lactating females, a few were breeding females and the balance were young males. The donors were killed with massive doses of nembutal and the blood secured from the thoracic cavity with a syringe, diluted with warm distilled water and injected

as quickly as possible. The blood of each animal was used individually and not pooled. Following injection into subcutaneous tissue of the back, the blood was spread under the skin with slight pressure. There were 118 mice in the experimental group and 111 in the control group at the time that the first tumor appeared. Animals dying before this age are not included.

Results.—In the control group of 111 females, 11 carcinomas of the mammary glands have appeared at an average age of 351.3 days; 100 mice have died tumor free at an average age of 508.7 days.

In the experimental group of 118 mice, 36 had mammary carcinoma at an average age of 354.0 days; 82 died tumor free at an average age of 460.5 days.

All of the tumors were examined histologically and were found to be adenocarcinomas. There were several mammary gland infections which might grossly have been mistaken for mammary gland tumors. These appeared in both the control and the experimental groups.

There were more than three times as many tumors in the experimental as in the control group. The percentage difference between the two groups was 20.6 with a standard error of the difference of 5.34. An analysis of the difference gives the difference over the standard error of 3.9. The *P* value was 0.000096. The odds are only 1 to 10,390 that a random sample would give as great or greater deviation. From analysis of the data it was ascertained that there was no litter-mate correlation and thus it was justifiable to use the whole number for comparison.

MICE	NO. OF ANIMALS AT TUMOR AGE	NO. OF TUMORS	% OF TUMORS	AVERAGE AGE AT APPEARANCE OF TUMOR	NO. DYING, TUMOR FREE	AGE DYING, TUMOR FREE
Experimental	118	36	30.51	354.0	82	460.5
Control	111	11	9.91	351.3	100	508.7

$$20.60 \pm 5.34 \quad D/\sigma = 3.9 \quad P = 0.000096$$

Discussion.—In testing for the significance the question arises whether both groups should be considered from the same date although the first tumor appeared a little later in one group than in the other. The first tumor in the control group appeared at 204 days and in the experimental group at 144 days. The data were examined by starting the control and experimental tabulations at the time of the earliest tumor of either group, that is, at 144 days, and again by starting each tabulation when the first tumor of that group appeared. The results remained significant with either method of calculation.

It is evident that the factor need not come only from breeding or from lactating females, as is shown from the age and sex of the animals used as donors. About four-fifths of the experimental mice received blood from males or immature females. Blood from the immature, non-lactating females, as well as from males, increased the incidence of breast carcinoma.

The blood injection did not return the incidence of mammary carcinoma to the high level of tumor incidence occurring in the Jackson Laboratory C3H strain itself. Instead of the 30% tumor incidence there should have been an incidence of 80% or more had this been so. It might be useful to hypothesize that that age of donor or recipient had something to do with this result. Another possibility is that the amount of blood injected was not sufficient to return the animals to the former high level of incidence. In line with this idea, Bittner, using fostered A strain mice, found that with the feeding of 0.9 cc. of milk 3 out of 10 developed mammary tumors, while with 1.7 cc. of milk, 8 out of 10 developed tumors. If the blood contained the influence in a quantity approximately equal to that in the milk, the 0.5 cc. of whole blood would not be expected to return the tumor incidence to the normal high level. There is some further evidence for interpretation of the data on a quantitative basis. Andervont has shown that high tumor young, suckling their own mothers for 17 hours or less, have a significantly lower mammary tumor incidence following foster nursing by low tumor females than young which have suckled for 24 to 48 hours. Twenty-eight mice suckling less than 17 hours had a tumor incidence of 25% while 23 sucking 24 to 48 hours had an incidence of 69.6%.

Other Tumors.—The second most frequent tumor to appear has been lymphoid leukemia which has now appeared in 25 animals. Fifteen of these were in the control group and at an average age of 585.4 days. Ten were in the treated group at an average age of 543.5 days. The general incidence, 10.1%, is a higher percentage of lymphatic tumors than has usually been recorded for the C3H strain. Appearing at the average age of 568.6 days it is evident that normally many animals which might have produced them were probably eliminated by the earlier appearing mammary gland tumors. Their appearance seems to have no relation to this experimental procedure except the fact that in general the mammary gland tumor incidence was fairly low thus leaving more than a normal proportion of animals to reach the upper age groups.

Summary.—Jackson Laboratory C3H mice which have had their tumor incidence lowered by foster nursing were injected at 1–3 months of age with 0.5 cc. of whole blood from normal high tumor C3H lactating females, young females and males. Significant differences are present between the inoculated mice and their litter-mate controls.

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DETERMINATION OF THE SUBGROUPS OF SMALL INDEX

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It is often less difficult to determine all the subgroups of a given group G which are of a small index, or of a relatively large order, than to determine all those of a small order contained in the group, in view of the fact that the number of the latter is often much larger than the number of the former.¹ In particular, the number of the subgroups of index 2 contained in G is always of the form $2^m - 1$ and each of these subgroups involves all the operators of odd order found in G as well as the commutator subgroup of G . When G is the symmetric group of degree n it is well known that it contains one and only one subgroup of index 2 for every value of n while when G is the alternating group of this degree it never contains any such subgroup. The operators of odd order contained in any group either generate the entire group or they generate an invariant subgroup with respect to which the order of the quotient group is a power of 2.

A group cannot contain as many subgroups of index 2 as it has operators and when it has just one less such subgroup it is of order 2^m , abelian, and of type 1. All other groups of order g contain less than $g - 1$ subgroups of index 2 and at most $g/2 - 1$ such subgroups. If G contains this latter number of subgroups of index 2 then their cross-cut is of order 2 and G involves operators of order 4 each of whose squares generates this cross-cut. All its cyclic subgroups of order 4 are invariant since they involve the commutator subgroup. If G contains an invariant operator of order 4 then exactly half of its operators are of this order since the products of this operator into its other operators of the same order are either of order 2 or they are the identity, while the products of this operator into the remaining operators of the group are all of order 4. If G contains no invariant operator of order 4 then it contains a subgroup of index 2 composed of its operators which are commutative with one of its operators of this order. Hence there results the following theorem: *If a group of order g contains as many as $g/2 - 1$ subgroups of index 2 its order is of the form 2^m , and if it involves operators of order 4 then at least one-fourth and at most three-fourths of its operators are of this order and all these operators have a common square.*

The number of the subgroups of index z in G is always $g/k - 1$, where k is the order of the cross-cut of all these subgroups. A necessary and sufficient condition that the order of G is a power of 2 is that k is a power of 2. The largest number of subgroups of index 2 in a group whose order is not a power of 2 is therefore $g/3 - 1$. For instance, two of the five groups of order 12 separately contain three subgroups of order 6. One of

these groups is abelian and the other is dihedral. A necessary and sufficient condition that a group contains at least one subgroup of index 2 is that it is not generated by the operators of odd order contained in it since these operators generate an invariant subgroup with respect to which the order of the quotient group is a power of 2, as was noted above.

A subgroup of prime index p contained in G is not necessarily invariant when p is odd and the number of such invariant subgroups in G cannot exceed $(p^\lambda - 1)/(p - 1)$, when p^λ is the highest power of p which divides the order of G . This results directly from the fact that the cross-cut of such invariant subgroups involves the p th power of every operator of G as well as the commutator subgroup of G . If a subgroup of index p is non-invariant under G it belongs to set of p conjugate subgroups which are transformed under G according to a transitive permutation group of degree p and hence G contains an invariant subgroup which corresponds to the identity of this permutation group. In particular, the only symmetric or alternating groups which contain a subgroup of index 3 are those of degrees 3 and 4.

Suppose that the group G contains both an invariant and a non-invariant subgroup of the same prime index p . It was noted above that it will then contain at least one set of p conjugate subgroups which are transformed under it according to a transitive permutation group of degree p with which G is isomorphic. The invariant subgroup of index p which is supposed to be contained in G cannot be simply isomorphic with an invariant proper subgroup of the given transformation group since such a proper subgroup would involve all the permutations of order p contained in this transformation group and hence it could not be of index p under G since the order of this transformation group cannot be divisible by p^2 . It therefore results that the given invariant proper subgroup corresponds to all the operators of this transformation group and hence it also contains p conjugate subgroups, p being an odd prime number, and that the order of G is divisible by p^2 . Hence there results the following theorem: *If a group contains an invariant subgroup of odd prime index p and also p conjugate subgroups of this index, then the order of the group is divisible by the square of p and this invariant subgroup also contains a set of p conjugate subgroups.*

When G contains a non-invariant subgroup of index p and also an invariant subgroup of a different prime index q , then p must exceed q . In fact, if p were less than q , then G would be isomorphic with a non-regular permutation group of degree p and every invariant subgroup of such a permutation group must involve all its operators of order p and hence it corresponds to a cyclic quotient group whose order divides $p - 1$. The invariant subgroup of index q in G could therefore not correspond to an invariant proper subgroup of this group. It therefore results that p must exceed q and that q must divide $p - 1$. That is, *when a group contains an*

invariant subgroup of prime index and a non-invariant subgroup of a different prime index, then the former of these primes must divide the latter diminished by unity.

If the index of a subgroup of G is composite, its smallest possible value is 4. If G contains invariant subgroups of index 4 the total number of these subgroups has a cross-cut with respect to which the quotient group is abelian and involves no operator whose order exceeds 4. Hence the number of its invariant subgroups of index 4 is equal to the number of subgroups of index 4 in such an abelian group whose order is a power of 2. The given cross-cut involves the commutator subgroup of G since a group of order 4 is necessarily abelian and the order of the quotient group which corresponds to this cross-cut is a divisor of a Sylow subgroup of G whose order is a power of 2. The only symmetric or alternating group which contains a subgroup of index 4 is of degree 4. It is well known that the smallest index of a subgroup of a symmetric or alternating group increases indefinitely as the degree of this group increases indefinitely. The fact that this index could not be less than the degree when this degree exceeds 4 results directly from the fact that the symmetric or the alternating group whose degree exceeds 4 cannot contain an invariant proper subgroup whose index exceeds 2 and this is only possible in the former case.

When G contains a non-invariant subgroup of prime index p it also contains a set of p conjugate subgroups since this subgroup cannot be transformed into itself by any operators of G except those which appear in it but when G contains a non-invariant subgroup of composite index k it is possible for every value of k to construct a group which does not contain k conjugate subgroups of this index. In fact, this is true of every imprimitive group in which the subgroup composed of all the permutations which omit one letter is regular on a number, greater than unity, of letters which divide k . In particular, the octic group contains non-invariant subgroups of index 4 but it does not contain a set of four conjugate subgroups of order 2. It is not possible for a group to have both an invariant subgroup of index 4 and also a complete set of four conjugate subgroups of this index unless the invariant subgroup also contains four conjugate subgroups of index 4 and hence is isomorphic either with the alternating group of degree 4 or with the symmetric group of this degree.

It was observed very early in the development of permutation groups that the symmetric group, as well as the alternating group, of degree 6 contains transitive subgroups of index 6. It may be noted in this connection that whenever a symmetric or an alternating group of degree n contains more than one set of conjugate subgroups of index n then all of these sets except one must be composed of transitive subgroups of degree n . This results directly from the fact that if such subgroups were intransitive then the corresponding symmetric or alternating group would involve

more than one invariant proper subgroup. This is known not to be the case when the degree of the group exceeds 4, and the theorem is known to be true as regards the groups whose degrees are less than 5. Similar considerations as regards the symmetric or the alternating group of a prime degree exhibit the fact that such a group cannot contain more than one set of conjugate subgroups of index n but this fact is included in a more general theorem which will be proved in the following paragraph.

When a transitive unique permutation group of degree n , in which the subgroups composed of all the permutations which omit one letter are of degree $n - 1$, contains other subgroups of index n which involve no invariant proper subgroup of the entire group then this permutation group admits outer automorphisms and vice versa. This theorem results directly from the fact that this permutation group can be represented as a transitive group of degree n in which such other subgroups are the subgroups composed of all the permutations which omit one letter and hence the group admits an automorphism in which one of these second set of subgroups corresponds to one of the original subgroups which are composed of all the permutations which omit one letter. From this theorem it results directly that no alternating or symmetric group of degree n , when n is not 6, contains more than one set of conjugate subgroups of index n since the group of automorphisms of these groups is known to be the corresponding symmetric group. The connection between automorphisms and the existence of subgroups should be emphasized in this connection. From this theorem it follows directly that the simple group of order 168 admits outer automorphisms, as has also been proved otherwise.

In the *Theory of Group Characters* by D. E. Littlewood, page 147 (1940), it is stated that "the easiest subgroups to find are those with large orders, and these present most difficulty by other methods." The present article implies that this is not always the

EXISTENCE OF PERIODIC SOLUTIONS FOR CERTAIN DIFFERENTIAL EQUATIONS

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1. In the present Note we prove the existence of periodic solutions for the equation

$$\frac{d^2x}{dt^2} + g'(x)\frac{dx}{dt} + f(x) = e(t), \quad (1)$$

where $e(t)$ has the period T , and g, f are restricted as stated below. As we shall see, the proof is essentially elementary. The type of equation under discussion generalizes the equation for the response of an electrical series circuit with resistance R , capacity C (both constant) and an inductor with current-flux saturation curve $i = h(\varphi)$. Here (1) is the differential equation of the flux with

$$f = \frac{h}{C}, g(x) = Rh.$$

The function $h(x)$ may be satisfactorily represented by an odd polynomial such that $xh(x) > 0$. This particular case suffices to indicate the importance of the periodic solutions of (1).

THEOREM 1. *The following are sufficient conditions in order that (1) possess a periodic solution of period T :*

I. The derivatives $e'(t), f'(x), g'(x)$ exist for all values of their variables.

II. $e(t)$ has the period T .

III. $\frac{f(x)}{x} \rightarrow +\infty$ with $|x|$.

IV. There exist $b, B > 0$ such that

$$|g(x) - bf(x)| \leq B \cdot |x|.$$

If we set for convenience

$$\frac{f(x)}{x} = F(x), \frac{g(x)}{x} = G(x),$$

then III, IV may be replaced by:

III'. $F(x) \rightarrow +\infty$ with $|x|$. (Hence, by IV, $G(x)$ has the same property.)

IV'. $|G(x) - bF(x)| \leq B$.

2. *Remark 1.* Condition I is merely designed to guarantee the existence and uniqueness of a solution with any given initial values of x and $\frac{dx}{dt}$. It would be possible to replace I by a weaker but less easily described condition.

Remark 2. The restrictions I, ..., IV are manifestly fulfilled by the equation of the series circuit, but not, for instance, by van der Pol's equation, or generally by the relaxation equation¹ with or without periodic disturbance.

Remark 3. Chevalley has proved the existence of periodic solutions for equations (1) with f, g still less restricted than ours. His argument rests likewise upon an analogue of Lemma 1 applied to the phase space (x, v) with ellipses replaced by the curves

$$2u = y^2 + 2k(x), \quad k = \int_0^x f(x) dx.$$

However we understand that the proof based on elliptic ovals given below is far simpler than his.

3. *Method.* If we set

$$\frac{dx}{dt} + g(x) = y, \tag{2}$$

then the solution of (1) is equivalent to that of the system consisting of (2) and of

$$\frac{dy}{dt} + f(x) = e(t), \tag{3}$$

and our theorem will follow if we can show that the system possesses a solution (x, y) periodic and of period T .

Consider now any point $P(x, y)$. According to the classical existence theorems there is a unique solution or *trajectory* $\Gamma(x(t), y(t))$ such that $x(0) = x_0, y(0) = y_0$. If Q is the point $(x(T), y(T))$ then $P \rightarrow Q$ defines a mapping S of the plane xy into itself and Theorem 1 will follow if we can prove that S has a fixed point. For, owing to the periodicity of $e(t)$, the trajectory Γ is likewise uniquely defined by $x(T) = x_0, y(T) = y_0$. Hence if Γ returns to P at time T , it is necessarily periodic.

By Brouwer's fixed point theorem then our theorem will follow from

Lemma 1. *There is a closed two-cell mapped into itself by S .*

We will prove in fact the more precise

Lemma 2. *There is a region bounded by an ellipse which is mapped into itself by S .*

4. Consider the definite quadratic form

$$2u = ax^2 - 2xy + by^2, \quad ab > 1, \quad a > 0. \quad (4)$$

Along a trajectory we have

$$u' = \frac{du}{dt} = (ax - y)(y - xG) + (by - x)(e - xF). \quad (5)$$

Let r, θ be polar coördinates for the plane xy . We first show that Lemma 2 is a consequence of

Lemma 3. When r is above a certain value then $u' < 0$.

In fact assume Lemma 3 to hold for $r > r_0$. Since the distance of the ellipse (4) from the origin $\rightarrow +\infty$ with u , we may choose u such that it exceeds r_0 , and then Lemma 2 will follow from Lemma 3. Thus everything reduces to proving the latter. The proof will be divided into two parts.

(a) We first take an α between 0 and $\frac{\pi}{2}$ and assume $\left|\theta - \frac{\pi}{2}\right|$ or $\left|\theta - \frac{3\pi}{2}\right| \leq \alpha$. If $|x|$ is sufficiently large both G and F are positive, and hence in view of $ab > 1$, for $|x|$ large enough

$$aG - F > \frac{1}{b}(G - bF) > -\frac{B}{b}.$$

Hence there is a positive C such that whatever x we have

$$aG - F > -C$$

and therefore

$$\frac{u'}{r^2} < \{C \cos^2 \theta + (a + B) |\sin \theta| |\cos \theta| - \sin^2 \theta\} - \frac{e}{r}(b \sin \theta - \cos \theta).$$

The bracket is a continuous function of θ whose value is -1 for $\theta = \frac{\pi}{2} + k\pi$.

Therefore for $|\cos \theta|$ sufficiently small, i.e., choosing α sufficiently small, the bracket will be as near -1 as we please. Since e is bounded we may choose

r_1 such that for $r > r_1$ the term $-\frac{e}{r}(b \cos \theta - \sin \theta)$ is arbitrarily small in absolute value. Hence we may choose α, r_1 such that $u' < 0$ under the conditions considered.

(b) The point (x, y) is such that $\left|\theta - \frac{\pi}{2}\right|$, and $\left|\theta - \frac{3\pi}{2}\right| > \alpha$, where α is the angle just selected. Evidently

$$e = \frac{e(b \sin \theta - \cos \theta)}{r \cos^2 \theta}$$

is bounded under the conditions here considered. Now

$$\frac{u'}{r^2} = -(aG - F + \epsilon) \cos^2 \theta + (a + G - bF) \sin \theta \cos \theta - \sin^2 \theta \quad (7)$$

is a quadratic form in $\sin \theta, \cos \theta$ whose discriminant

$$4(aG - F + \epsilon) - (a + G - bF)^2 > 4(ab - 1)F + \text{const.} \rightarrow +\infty \text{ with } |x|.$$

Therefore the form in (7) is definite negative, and so $u' < 0$ if θ is as stated, and if $r > r_2$, where r_2 is chosen large enough.

We conclude then that Lemma 3 holds for $r > \max. (r_1, r_2)$ and so Theorem 1 is proved.

5. *Generalization.* Elliptic or other algebraic ovals may be utilized in many other cases to prove the existence of periodic solutions. Thus consider the system

$$\frac{dx_i}{dt} = X_i(x_1, \dots, x_n, t) + Y_i(x_1, \dots, x_n) \quad (8)$$

where X_i, Y_i are polynomials in the x_j with degree $X_i < M = \text{degree } Y_i$ and the coefficients of X_i are bounded periodic functions of period T . If there exists a positive definite quadratic form

$$2u = \sum a_{ij} x_i x_j$$

such that along a trajectory

$$u' = \sum a_{ij} x_i Y_j < 0$$

for $r^2 = \sum x_i^2$ sufficiently large, then the system (8) possesses a periodic solution.

6. Let us observe finally that instead of $u' < 0$ for r sufficiently large we could merely ask that its sign be constant. For if u increases with t , it decreases with $-t$, and since the transformation $t \rightarrow -t$ does not affect periodic solutions, u increasing with t would be acceptable throughout.

¹ See the recent paper by Norman Levinson and O. K. Smith, *Duke Math. Jour.*, **9**, 382-403 (1942), where a number of earlier references, notably to van der Pol, von Kármán and Liénard are given.

ON THE EUCLIDEAN CONNECTIONS IN A FINSLER SPACE

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The generalization of the parallelism of Levi-Civita in a Riemann space to a Finsler space has been regarded as one of the most important problems of Finslerian Geometry. For its solution different suggestions were made by J. L. Synge,¹ J. H. Taylor,² L. Berwald³ and E. Cartan.⁴ In this note we shall study the problem by employing a different method—the method of equivalence. We shall prove that in a general Finsler space an infinite number of Euclidean connections can be defined, in which the connections defined by other authors are included as particular cases.

Let x^i be the coordinates of an n -dimensional Finsler space, whose fundamental integral is

$$s = \int_{t_0}^{t_1} F\left(x^i, \frac{dx^i}{dt}\right) dt, \quad (1)$$

where F is positively homogeneous of the first order in the last n arguments:

$$F(x^i, \lambda y^i) = \lambda F(x^i, y^i), \quad y^i = \frac{dx^i}{dt}, \quad \lambda > 0. \quad (2)$$

It is well known that the Pfaffian form

$$\omega = \frac{\partial F}{\partial y^i} dx^i \quad (3)$$

is invariant (under a general point transformation). We adjoin to the coordinates x^i of the space $n(n-1)$ auxiliary variables v_k^α , subjected to the conditions

$$v_k^\alpha y^k = 0, \quad (4)$$

and put

$$v_k^\alpha = \frac{\partial F}{\partial y^k}. \quad (5)$$

Then in the space of all the variables x^i, y^i, v_k^α we have n linearly independent invariant Pfaffian forms, namely,

$$\omega^i = v_k^i dx^k, \quad (6)$$

where $\omega^n = \omega$. It is from the Pfaffian forms ω^i that we shall develop our invariant theory of Finsler spaces, from which the Euclidean connections in the space are derived as consequences.

We introduce the elements u_k^i of the inverse matrix of (v_k^i) , so that we have

$$u_j^i v_k^j = v_j^i u_k^j = \delta_k^i. \quad (7)$$

The elements u_n^k do not depend on the auxiliary variables, since a comparison of equations (4), (5), (7) gives

$$u_n^k = \frac{1}{F} y^k. \quad (8)$$

If we form the exterior derivative of ω^n , we see that we can write

$$(\omega^n)' = [\omega^\alpha \omega_\alpha^n], \quad (9)$$

where

$$\begin{aligned} \omega_\alpha^n = & -u_\alpha^k \frac{\partial^2 F}{\partial y^j \partial y^k} dy^j + \frac{1}{F} u_\alpha^j \left(\frac{\partial F}{\partial x^j} - \frac{\partial^2 F}{\partial x^k \partial y^j} y^k \right) \omega^n + \\ & u_\alpha^j u_\beta^k \frac{\partial^2 F}{\partial x^j \partial y^k} \omega^\beta + \lambda_{\alpha\beta} \omega^\beta, \quad \lambda_{\alpha\beta} = \lambda_{\beta\alpha}, \end{aligned} \quad (10)$$

the $n(n-1)/2$ quantities $\lambda_{\alpha\beta}$ being arbitrary.

We shall suppose that the fundamental integral (1) leads to a "regular problem" of the calculus of variations. As is well known, this amounts to assuming that the matrix

$$\left(\frac{\partial^2 F}{\partial y^i \partial y^j} \right)$$

is of rank $n-1$, or, in our notation, that the Pfaffian forms $\omega^i, \omega_\alpha^n$ are linearly independent.

If we form the exterior derivative of ω^α , we see that the following invariant conditions can be imposed:

$$(\omega^\alpha)' \equiv \delta^{\alpha\beta} [\omega_\beta^n \omega^n], \text{ mod. } \omega^\gamma, \quad (11)$$

where $\delta^{\alpha\beta}$ is Kronecker's symbol. The conditions (11) are equivalent to

$$u_\alpha^j u_\beta^k \left(F \frac{\partial^2 F}{\partial y^j \partial y^k} \right) = \delta_{\alpha\beta}, \quad (12)$$

under which the variables u_α^i are not all independent. By carrying out the calculation of $(\omega^\alpha)'$, we get

$$(\omega^\alpha)' - \delta^{\alpha\beta} [\omega_\beta^n \omega^n] = [\omega^\beta \omega_\beta^\alpha], \quad (13)$$

where

$$\omega_\beta^\alpha = v_k^\alpha du_\beta^k - \delta^{\alpha\gamma} \left(u_\gamma^j u_\beta^k \frac{\partial^2 F}{\partial x^j \partial y^k} + \lambda_{\beta\gamma} \right) \omega^n + \mu_{\beta\gamma}^\alpha \omega^\gamma, \quad (14)$$

the quantities $\mu_{\beta\gamma}^\alpha$ being symmetric in β, γ and being introduced to get the most general expression for $\omega^{\beta\alpha}$.

Since the variables u_α^i are connected by the relations (12), the Pfaffian forms ω_β^α are not linearly independent. In fact, we find that they satisfy the relations

$$\omega_{\alpha\beta} + \omega_{\beta\alpha} \equiv 0, \text{ mod. } \omega^i, \omega_\alpha^n,$$

where the quantities $\delta_{\alpha\beta}$ are used to raise and lower indices, thus

$$\omega_{\alpha\beta} = \delta_{\beta\gamma} \omega_\alpha^\gamma, \quad \omega_\alpha^\beta = \delta_\alpha^\gamma \omega_\gamma^\beta, \text{ etc.} \quad (15)$$

Our fundamental result is that we can, by a proper choice of $\lambda_{\alpha\beta}$, $\mu_{\beta\gamma}^\alpha$, arrive at the conditions

$$\omega_{\alpha\beta} + \omega_{\beta\alpha} = H_{\alpha\beta}^\gamma \omega_\gamma^n, \quad (16)$$

and that under these conditions the quantities $\lambda_{\alpha\beta}$, $\mu_{\beta\gamma}^\alpha$, and hence the Pfaffian forms ω_α^n , ω_β^α are completely determined. These Pfaffian forms are therefore invariant Pfaffian forms. The quantities $H_{\alpha\beta}^\gamma$ constitute the first set of invariants of the Finsler space. Their vanishing signifies that the Finsler space is a Riemann space.

The expressions for the exterior derivatives of ω_β^α , ω_α^n will lead to further invariants of the space. To find them we write the equations (9), (13), (16) in the condensed form

$$\left. \begin{aligned} (\omega^i)' &= [\omega^j \omega_j^i], \\ \omega_{ij} + \omega_{ji} &= H_{ijk} \omega^k, \end{aligned} \right\} \quad (17)$$

with the understanding that H_{ijk} is zero when any one of its indices is n . Putting

$$\Omega_k^i = (\omega_k^i)' - [\omega_k^j \omega_j^i], \quad (18)$$

we find, by simply applying the "theorem of Poincaré" that the exterior derivative of the exterior derivative of a Pfaffian form is zero, that Ω_k^i are of the form

$$\Omega_k^i = R_{k \cdot j l}^i [\omega^l \omega_j^j] + P_{k \cdot j}^{i \alpha} [\omega_\alpha^n \omega_j^j], \quad (19)$$

where

$$R_{k \cdot j l}^i + R_{k \cdot l j}^i = 0 \quad (20)$$

For a function F in our variables its differential dF is a linear combination of ω^i , ω_j^i , the coefficients of the linear combination being the "covariant derivatives." The invariants H_{ijk} , $R_{k \cdot j l}^i$, $P_{k \cdot j}^{i \alpha}$ and their covariant derivatives form a complete system of invariants in the sense that they are sufficient to determine a Finsler space up to a point transformation.

Now we shall enter into the geometrical interpretation of our results. For this purpose we put

$$\pi_{ij} = \omega_{ij} + \gamma_{ij\alpha} \omega^\alpha, \quad (21)$$

and impose the conditions

$$\gamma_{ij\alpha} + \gamma_{jia} = -H_{ija}, \quad (22)$$

in order to have

$$\pi_{ij} + \pi_{ji} = 0. \quad (23)$$

We also put

$$\pi^i = \omega^i. \quad (24)$$

The Pfaffian forms π^i, π_j^i , of which the latter have not yet been completely determined, will then be employed to define the Euclidean connection in the space.

To each set of variables x^i, y^i, v_k^α we attach a Euclidean space of n dimensions with the frame of reference $\overrightarrow{Me_1} \dots \overrightarrow{e_n}$, where M is a point and $\overrightarrow{e_1}, \dots, \overrightarrow{e_n}$ are n mutually perpendicular unit vectors through M . The equations

$$\left. \begin{aligned} dM &= \pi^i \overrightarrow{e_i} \\ d\overrightarrow{e_i} &= \pi_i^j \overrightarrow{e_j} \end{aligned} \right\} \quad (25)$$

then determine the infinitesimal displacement between two neighboring Euclidean spaces or a Euclidean connection. The property of the Euclidean connection depends on the expressions for the following exterior quadratic differential forms

$$\left. \begin{aligned} \Pi^i &= (\pi^i)' - [\pi^j \pi_j^i] \\ \Pi_j^i &= (\pi_j^i)' - [\pi_j^k \pi_k^i] \end{aligned} \right\} \quad (26)$$

And we find

$$\left. \begin{aligned} \Pi^i &= \Omega^i - \gamma_j^{i\alpha} [\omega^j \omega_\alpha^n], \\ \Pi_j^i &= \Omega_j^i + \gamma_j^{i\alpha} \Omega_\alpha^n - \gamma_j^{\alpha\beta} \gamma_\alpha^{i\rho} [\omega_\beta^n \omega_\rho^n] + [(d\gamma_j^{i\alpha} + \gamma_j^{i\beta} \omega_\beta^\alpha - \gamma_\beta^{i\alpha} \omega_j^\beta + \gamma_j^{\beta\alpha} \omega_\beta^i) \omega_\alpha^n]. \end{aligned} \right\} \quad (27)$$

Due to a reason which we shall give later, it is important to impose the condition that Π^i, Π_j^i be exterior quadratic differential forms in $\omega^i, \omega_\alpha^n$. This gives

$$d\gamma_j^{i\alpha} + \gamma_j^{i\beta} \omega_\beta^\alpha - \gamma_\beta^{i\alpha} \omega_j^\beta + \gamma_j^{\beta\alpha} \omega_\beta^i \equiv 0, \text{ mod. } \omega^k, \omega_\alpha^n. \quad (28)$$

Summing up, the conditions on $\gamma_{ij\alpha}$ are (22), (28). We shall satisfy (22) by supposing

$$\left. \begin{aligned} \gamma_{in\alpha} &= \gamma_{ni\alpha} = 0, \\ \gamma_{p\sigma\alpha} + \gamma_{\sigma p\alpha} &= -H_{p\sigma\alpha} \end{aligned} \right\} \quad (29)$$

Then (28) is reduced to the following more symmetrical form:

$$d\gamma_{\rho\sigma\alpha} + \gamma_{\rho\sigma\beta}\omega_{\alpha}^{\beta} + \gamma_{\beta\sigma\alpha}\omega_{\rho}^{\beta} + \gamma_{\rho\beta\alpha}\omega_{\sigma}^{\beta} \equiv 0, \text{ mod. } \omega^i, \omega_{\alpha}^n. \quad (30)$$

It is important to note that $\gamma_{\rho\sigma\alpha}$ have naturally to be invariants. An example of such a set of invariants is furnished by $H_{\rho\sigma\alpha}$. We can easily verify that the condition (30) for $\gamma_{\rho\sigma\alpha}$ is equivalent to saying that $\gamma_{\rho\sigma\alpha}$ is of the form

$$\gamma_{\rho\sigma\alpha} = G_{ijk}u_{\rho}^iu_{\sigma}^ju_{\alpha}^k, \quad (31)$$

where G_{ijk} are functions of x^m, y^m only.

To each set of invariants $\gamma_{\rho\sigma\alpha}$ satisfying the conditions (29), (30) we have the set of Pfaffian forms π^i, π_i^j . With these Pfaffian forms the equations (25) define a Euclidean connection, which gives the infinitesimal displacement between the Euclidean spaces attached to two neighboring sets of variables x^i, y^i, v_k^{α} . Owing to the property that Π^i, Π_j^i are exterior quadratic differential forms in $\omega^i, \omega_{\alpha}^n$, it follows that Π^i, Π_j^i are zero, when x^i, y^i are given as functions of a parameter t :

$$x^i = x^i(t), y^i = y^i(t). \quad (32)$$

Hence along a one-parameter family of contact elements (x^i, y^i) the system of equations (25) is completely integrable and the Euclidean spaces attached can be developed in one and the same Euclidean space. This is essentially the generalization of the well-known theorem of Fermi to a Finsler space. From this fact we see that we can regard the Finsler space as formed by the $(2n - 1)$ -parameter family of its contact elements and define a Euclidean connection in the space, with a "tangent Euclidean space" attached to each contact element. There are as many Euclidean connections in the space as there are invariants satisfying our conditions. If we take

$$\gamma_{\rho\sigma\alpha} = -1/2 H_{\rho\sigma\alpha}, \quad (33)$$

we get the Euclidean connection defined by Cartan.

In conclusion, we remark that each of our Euclidean connections has the property that the equivalence of the Euclidean connection is a necessary and sufficient condition for the equivalence of the Finsler spaces (under point transformations).

¹ Synge, J. L., *Trans. Amer. Math. Soc.*, **27**, 61-67 (1925).

² Taylor, J. H., *Ibid.*, **27**, 246-264 (1925).

³ Berwald, L., *Atti Congr. Mat., Bologna*, **4**, 263-270 (1928).

⁴ Cartan, E., *Les espaces de Finsler*, Paris, 1934.

⁵ It is agreed, throughout this paper, that a Latin index runs from 1 to n and a Greek index from 1 to $n - 1$.

A GENERALIZATION OF THE PROJECTIVE GEOMETRY OF LINEAR SPACES

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The projective geometry in a space of n dimensions may be briefly described as the geometry of the points and the straight lines in the space. For $n > 2$ the straight lines can be replaced by the linear spaces of a fixed dimension r , $1 \leq r \leq n - 1$. We have, in fact, the theorem that a one-to-one point transformation in the space, which carries the linear spaces of dimension r into themselves, is a projective transformation. The number of parameters on which the linear spaces of dimension r depend is equal to $N = (r + 1)(n - r)$. The geometry in a space of n dimensions in which there is given a family of r -dimensional varieties depending on N parameters is therefore in a certain sense a generalization of projective geometry. In this note we shall show that in such a space a projective connection can be defined. The geometry of paths¹ is a particular case of this geometry for $r = 1$, while the case $r = n - 1$ has been studied by M. Hachtroudi²

Let x^1, \dots, x^n be the coördinates in the space and let the family of varieties be defined by a completely integrable Pfaffian system of the form³

$$\left. \begin{aligned} dx^i - p_\alpha^i dx^\alpha &= 0, \\ dp_\alpha^i - r_{\alpha\beta}^i dx^\beta &= 0, \end{aligned} \right\} \quad (1)$$

where $r_{\alpha\beta}^i$ are functions of $x^\alpha, x^i, p_\alpha^i$. Before defining the projective connection in question, we shall develop the invariant theory of the family of varieties under non-singular point transformations

$$\left. \begin{aligned} x^{1*} &= x^1(x^1, \dots, x^n) \\ &\dots\dots\dots \\ x^{n*} &= x^n(x^1, \dots, x^n). \end{aligned} \right\} \quad (2)$$

The left-hand members of (1) are not invariant Pfaffian forms. To derive invariants or invariant Pfaffian forms of the family of varieties the following device will be repeatedly applied: We adjoin to the variables $x^\alpha, x^i, p_\alpha^i$ the new variables $u_j^\alpha, u_\beta^\alpha, u_j^i, u_{\alpha j}^{i\beta}, u_{\alpha j}^i$. Then the Pfaffian forms

$$\begin{aligned} \omega^\alpha &= u_\beta^\alpha dx^\beta + u_j^\alpha (dx^j - p_\beta^j dx^\beta), \\ \omega^i &= u_j^i (dx^j - p_\beta^j dx^\beta), \\ \omega_\alpha^i &= u_{\alpha j}^{i\beta} (dp_\beta^j - r_{\beta\gamma}^j dx^\gamma) + u_{\alpha j}^i (dx^j - p_\beta^j dx^\beta) \end{aligned} \quad (3)$$

are invariant (in the space of all the variables). The number of the new variables can sometimes be reduced by invariant conditions. In fact, we shall suppose

$$(\omega^i)' \equiv [\omega^\alpha \omega_\alpha^i], \text{ mod. } \omega^j, \quad (4)$$

which are equivalent to the conditions

$$u_{\alpha j}^{i\beta} = u_j^i v_\alpha^\beta, \quad (5)$$

v_α^β being defined by the relations

$$u_\alpha^\gamma v_\gamma^\beta = v_\alpha^\gamma u_\gamma^\beta = \delta_\alpha^\beta. \quad (6)$$

Suppose the conditions (4) or (5) be satisfied. Then we can write, by introducing the new Pfaffian forms $\varphi_\beta^\alpha, \varphi_j^\alpha, \varphi_j^i$, the exterior derivatives of ω^α, ω^i in the forms

$$\left. \begin{aligned} (\omega^\alpha)' &= [\varphi_\beta^\alpha \omega^\beta] + [\varphi_j^\alpha \omega^j], \\ (\omega^i)' &= [\varphi_j^i \omega^j] + [\omega^\alpha \omega_\alpha^i]. \end{aligned} \right\} \quad (7)$$

The fact that the exterior derivatives of $(\omega^\alpha)', (\omega^i)'$ are zero gives

$$\left. \begin{aligned} [(\varphi_j^i)' - \varphi_k^i \varphi_j^k - \varphi_j^\alpha \omega_\alpha^i] \omega^j - [(\omega_\alpha^i)' + \varphi_\alpha^\beta \omega_\beta^i - \varphi_j^i \omega_\alpha^j] \omega^\alpha &= 0, \\ [(\varphi_j^\alpha)' - \varphi_\beta^\alpha \varphi_j^\beta - \varphi_k^\alpha \varphi_j^k] \omega^j + [(\varphi_\beta^\alpha)' - \varphi_\gamma^\alpha \varphi_\beta^\gamma + \varphi_j^\alpha \omega_\beta^j] \omega^\beta &= 0. \end{aligned} \right\} \quad (8)$$

The first equation shows that the expression $(\omega_\alpha^i)' + [\varphi_\alpha^\beta \omega_\beta^i] - [\varphi_j^i \omega_\alpha^j]$ contains ω^j or ω^β in each of its terms. Since the system

$$\omega^i = 0, \quad \omega_\alpha^i = 0 \quad (9)$$

is equivalent to (1) and is hence completely integrable, we see that we can put

$$(\omega_\alpha^i)' = -[\varphi_\alpha^\beta \omega_\beta^i] + [\varphi_j^i \omega_\alpha^j] + [\varphi_{\alpha j}^i \omega^j] + Q_{\alpha \gamma j}^{i\beta} [\omega_\beta^j \omega^\gamma], \quad (10)$$

where $\varphi_{\alpha j}^i$ are new Pfaffian forms and where

$$Q_{\alpha \gamma j}^{i\beta} = Q_{\gamma \alpha j}^{i\beta}. \quad (11)$$

Let us see whether $Q_{\alpha \gamma j}^{i\beta}$ are invariants. For this purpose notice that the Pfaffian forms $\varphi_\beta^\alpha, \varphi_j^i$ in (7) are determined up to the transformation

$$\left. \begin{aligned} \varphi_\beta^{\alpha*} &= \varphi_\beta^\alpha + a_{\beta\gamma}^\alpha \omega^\gamma + a_{\beta j}^\alpha \omega^j, & a_{\beta\gamma}^\alpha &= a_{\gamma\beta}^\alpha, \\ \varphi_j^{i*} &= \varphi_j^i + a_{jk}^i \omega^k, & a_{jk}^i &= a_{kj}^i, \end{aligned} \right\} \quad (12)$$

where the a 's are arbitrary. When $\varphi_\beta^\alpha, \varphi_j^i$ are replaced, respectively, by $\varphi_\beta^{\alpha*}, \varphi_j^{i*}$, the equation (10) retains its form, while $Q_{\alpha \gamma j}^{i\beta}$ are replaced by $Q_{\alpha \gamma j}^{i\beta*}$, related to $Q_{\alpha \gamma j}^{i\beta}$ as follows:

$$Q_{\alpha \gamma j}^{i\beta*} = Q_{\alpha \gamma j}^{i\beta} - \delta_{\alpha\gamma}^i a_{\alpha\gamma}^\beta.$$

This shows that $Q_{\alpha\gamma j}^{i\beta}$ are not invariants. We can, however, modify them such that the conditions

$$Q_{\alpha\gamma j}^{j\beta} = 0 \quad (13)$$

are satisfied. Under these conditions $Q_{\alpha\gamma j}^{i\beta}$ are invariants.

If we carry out the calculation of the expression for $Q_{\alpha\beta j}^{i\beta}$, we see that, by a proper choice of $u_{\alpha j}^i$, the conditions

$$Q_{\alpha\beta j}^{i\beta} = 0 \quad (14)$$

can be fulfilled. This determines $u_{\alpha j}^i$ in terms of x^α , x^i , p_α^i , u_j^i , u_β^α , and parameters or new variables which we denote by v_α . When $r = 1$ or $r = n - 1$, the conditions (13) and (14) signify that all $Q_{\alpha\gamma j}^{i\beta}$ vanish.

The conditions (13) and (14) have an effect on the expressions for $(\varphi_\beta^\alpha)'$, $(\omega_\alpha^i)'$. To find these expressions we apply exterior differentiation to (10), which gives

$$[\{\delta_j^i(-\varphi_\alpha^{\beta'} - \varphi_\alpha^\gamma \varphi_\gamma^\beta) + \delta_\alpha^\beta(\varphi_j^{i'} - \varphi_k^i \varphi_j^k) - \pi_{\alpha\gamma j}^{i\beta} \omega^\gamma - \varphi_{\alpha j}^i \omega^\beta + Q_{\alpha\gamma k}^{i\rho} Q_{\rho\sigma j}^{k\beta} \omega^\sigma \omega^\gamma\} \omega_\beta^j] \equiv 0, \text{ mod. } \omega^k,$$

where

$$\pi_{\alpha\gamma j}^{i\beta} = dQ_{\alpha\gamma j}^{i\beta} - Q_{\alpha\gamma j}^{k\beta} \varphi_k^i + Q_{\alpha\gamma k}^{i\beta} \varphi_j^k - Q_{\alpha\gamma j}^{i\rho} \varphi_\rho^\beta + Q_{\rho\gamma j}^{i\beta} \varphi_\alpha^\rho + Q_{\alpha\beta j}^{i\rho} \varphi_\gamma^\rho.$$

From (8) we get respectively

$$\begin{aligned} (\varphi_j^i)' - [\varphi_k^i \varphi_j^k] &\equiv [\varphi_j^\alpha \omega_\alpha^i] - [\varphi_\alpha^i \omega^\alpha], \text{ mod. } \omega^k, \\ (\varphi_\beta^\alpha)' - [\varphi_\gamma^\alpha \varphi_\beta^\gamma] + [\varphi_j^\alpha \omega_\beta^j] &\equiv [\theta_{\beta\gamma}^\alpha \omega^\gamma], \text{ mod. } \omega^k, \end{aligned}$$

where $\theta_{\beta\gamma}^\alpha$ are newly introduced Pfaffian forms. Substituting these expressions into the last equation, we shall get

$$\delta_j^i \theta_{\alpha\gamma}^\beta + \delta_\alpha^\beta \varphi_{\gamma j}^i + \delta_\gamma^\beta \varphi_{\alpha j}^i + \pi_{\alpha\gamma j}^{i\beta} \equiv 0, \text{ mod. } \omega^\alpha, \omega^i, \omega_\alpha^i.$$

The conditions (13) and (14) have as consequences

$$\pi_{\alpha\gamma j}^{j\beta} = 0, \quad \pi_{\alpha\beta j}^{i\beta} = 0,$$

so that the above equation gives, respectively,

$$(n - r) \theta_{\alpha\gamma}^\beta + \delta_\alpha^\beta \varphi_{\gamma j}^i + \delta_\gamma^\beta \varphi_{\alpha j}^i \equiv 0, \text{ mod. } \omega^\alpha, \omega^i, \omega_\alpha^i,$$

$$\varphi_{\alpha j}^i \equiv \frac{1}{n - r} \delta_j^i \varphi_\alpha^i, \text{ mod. } \omega^\alpha, \omega^i, \omega_\alpha^i,$$

where φ_α is an abbreviation of $\varphi_{\alpha j}^j$. It follows that the exterior derivatives of ω_α^i , φ_β^α are of the forms

$$(\omega_\alpha^i)' = -[\varphi_\alpha^\beta \omega_\beta^i] + [\varphi_j^i \omega_\alpha^j] + \frac{1}{n - r} [\varphi_\alpha \omega^i] + \Omega_\alpha^i, \quad (15)$$

$$(\varphi_\beta^\alpha)' \equiv [\varphi_\gamma^\alpha \varphi_\beta^\gamma] - [\varphi_j^\alpha \omega_\beta^j] - \frac{1}{n-r} \delta_\beta^\alpha [\varphi_\gamma \omega^\gamma] - \frac{1}{n-r} [\varphi_\beta \omega^\alpha] + R_{\beta\gamma\rho}^\alpha [\omega^\rho \omega^\gamma] + R_{\beta\gamma j}^{\alpha\rho} [\omega_\rho^j \omega^\gamma], \text{ mod. } \omega^i, \quad (16)$$

where

$$\Omega_\alpha^i = Q_{\alpha j\beta}^i [\omega^\beta \omega^j] + Q_{\alpha jk}^i [\omega^k \omega^j] + Q_{\alpha jk}^{i\beta} [\omega_\beta^k \omega^j] + Q_{\alpha\gamma j}^{i\beta} [\omega_\beta^j \omega^\gamma]. \quad (17)$$

It is to be noticed that the Pfaffian forms φ_β^α , φ_j^i , φ_j^α , φ_α introduced above are not invariant, because they are not completely determined by the equations (7), (15). These Pfaffian forms are determined up to a transformation which can easily be written down. By investigating the effect of this transformation on the coefficients in (15), (16), it is not difficult to show that we can choose the φ 's such that we have

$$Q_{\alpha j\beta}^i = 0, \quad Q_{\alpha jk}^{i\beta} = 0, \quad R_{\beta\gamma j}^{\alpha\gamma} = 0, \quad Q_{\beta jk}^{i\beta} = 0. \quad (18)$$

Under these conditions the Pfaffian forms in question are determined up to the transformation

$$\left. \begin{aligned} \varphi_\beta^{\alpha*} &= \varphi_\beta^\alpha + \frac{1}{n-r+1} \delta_\beta^\alpha a_j \omega^j, \\ \varphi_j^{\alpha*} &= \varphi_j^\alpha + \frac{1}{n-r+1} a_j \omega^\alpha + a_{jk}^\alpha \omega^k, \quad a_{jk}^\alpha = a_{kj}^\alpha, \\ \varphi_j^{i*} &= \varphi_j^i + \frac{1}{n-r+1} (\delta_j^i a_k \omega^k + a_j \omega^i), \\ \varphi_\alpha^* &= \varphi_\alpha + a_{\alpha j} \omega^j + \frac{n-r}{n-r+1} a_j \omega_\alpha^j, \end{aligned} \right\} \quad (19)$$

where the a 's are arbitrary.

We now take v_α , a_j to be new variables and adjoin them to the set of variables x^α , x^i , p_α^i , u_j^i , u_β^α , u_j^α . All these $n(n+2)$ variables then form the set which we shall deal with and by invariance we shall mean the invariance under a general transformation in these variables. It is in this sense that the Pfaffian forms φ_β^α , φ_j^i are invariant. In order to find a set of linearly independent invariant Pfaffian forms, whose number is equal to the number of variables, we form the exterior derivatives of φ_β^α , φ_j^i . From (8), (10) we see that we can set

$$\left. \begin{aligned} (\varphi_j^i)' - [\varphi_k^i \varphi_j^k] - [\varphi_j^\alpha \omega_\alpha^i] + \frac{1}{n-r} \delta_j^i [\varphi_\alpha \omega^\alpha] - Q_{\alpha j\beta}^i [\omega^\alpha \omega^\beta] - Q_{\alpha jk}^i [\omega^\alpha \omega^k] - Q_{\alpha jk}^{i\beta} [\omega^\alpha \omega_\beta^k] &= [\theta_{jk}^i \omega^k], \\ (\varphi_\beta^\alpha)' - [\varphi_\gamma^\alpha \varphi_\beta^\gamma] + [\varphi_j^\alpha \omega_\beta^j] + \frac{1}{n-r} \delta_\beta^\alpha [\varphi_\gamma \omega^\gamma] + \frac{1}{n-r} [\varphi_\beta \omega^\alpha] - R_{\beta\gamma\rho}^\alpha [\omega^\rho \omega^\gamma] - R_{\beta\gamma j}^{\alpha\rho} [\omega_\rho^j \omega^\gamma] &= [\theta_{\beta j}^\alpha \omega^j], \end{aligned} \right\} \quad (20)$$

where $\theta_{jk}^i, \theta_{\beta j}^\alpha$ are newly introduced Pfaffian forms, of which the former ones are subjected to the conditions

$$[\theta_{jk}^i \omega^j \omega^k] = 0. \quad (21)$$

We form the equation obtained by exterior differentiation of (15). A complete analysis of that equation will give the following two consequences:

$$\delta_k^i \theta_{\beta j}^\beta - r \theta_{kj}^i - \frac{1}{n-r} \delta_j^i (\theta_{\beta k}^\beta - r \theta_k) \equiv 0, \quad \text{mod. } \omega^\alpha, \omega^i, \omega_\alpha^i; \quad (22)$$

$$\varphi_\alpha' \equiv -[\varphi_\alpha^\beta \varphi_\beta] - [\theta_{\alpha k}^\beta \omega_\beta^k] + [\theta_k \omega_\alpha^k] - Q_{\alpha\gamma k}^{j\beta} [\varphi_j^\gamma \omega_\beta^k] + U_{\alpha\gamma k}^\beta [\omega_\beta^k \omega^\gamma] + U_{\alpha\beta\gamma} [\omega^\beta \omega^\gamma], \quad \text{mod. } \omega^i \quad (23)$$

where $\theta_k = \theta_{kj}^j$. On the other hand, by exterior differentiation of the second equation of (20), we can get

$$Q_{\beta\sigma j}^{k\alpha} \varphi_k^\sigma + \delta_\beta^\alpha \theta_{\gamma j}^\gamma - (r+1) \delta_\beta^\alpha \theta_j + (n+1) \theta_{\beta j}^\alpha \equiv 0, \quad \text{mod. } \omega^\beta, \omega^i, \omega_\alpha^i. \quad (24)$$

From (22) and (24) we then find

$$\theta_{\beta j}^\alpha \equiv \frac{1}{n-r+1} \delta_\beta^\alpha \theta_j - \frac{1}{n+1} Q_{\beta\sigma j}^{k\alpha} \varphi_k^\sigma, \quad \text{mod. } \omega^\alpha, \omega^i, \omega_\alpha^i, \quad (25)$$

$$\theta_{kj}^i \equiv \frac{1}{n-r+1} (\delta_j^i \theta_k + \delta_k^i \theta_j), \quad \text{mod. } \omega^\alpha, \omega^i, \omega_\alpha^i. \quad (26)$$

In this way we have introduced the Pfaffian forms θ_k , which involve da_k and are linearly independent from $\omega^\alpha, \omega^i, \omega_\alpha^i, \varphi_\beta^\alpha, \varphi_j^i, \varphi_j^\alpha, \varphi_\alpha$. The total number of linearly independent Pfaffian forms is now equal to the number of variables. But of these forms $\varphi_j^\alpha, \varphi_\alpha, \theta_k$ are not yet invariant. To derive invariant Pfaffian forms from them, further conditions are necessary. We put, according to (25), (26),

$$\left. \begin{aligned} [\theta_{jk}^i \omega^k] &= \frac{1}{n-r+1} (\delta_j^i [\theta_k \omega^k] + [\theta_j \omega^i]) + S_{jkl}^i [\omega^l \omega^k] + \\ &\quad S_{jkl}^i [\omega^\gamma \omega^k] + S_{jkl}^i [\omega_\gamma^l \omega^k], \\ [\theta_{\beta j}^\alpha \omega^j] &= -\frac{1}{n+1} Q_{\beta\sigma j}^{k\alpha} [\varphi_k^\sigma \omega^j] + \frac{1}{n-r+1} \delta_\beta^\alpha [\theta_j \omega^j] + \\ &\quad R_{\beta j \gamma}^\alpha [\omega^\gamma \omega^j] + R_{\beta j k}^\alpha [\omega^k \omega^j] + R_{\beta j k}^{\alpha\gamma} [\omega_\gamma^k \omega^j]. \end{aligned} \right\} \quad (27)$$

It is then easy to show that under the conditions

$$S_{kj\gamma}^j = 0, S_{kj\gamma}^i = 0, R_{\beta j \gamma}^\beta = 0, R_{\beta j k}^{\beta\alpha} + R_{\beta k j}^{\beta\alpha} = 0 \quad (28)$$

the Pfaffian forms $\varphi_j^\alpha, \varphi_\alpha$ are completely determined. The uniqueness of θ_k depends on the expression for $(\varphi_j^\alpha)'$. We find

$$\begin{aligned}
(\varphi_j^\alpha)' = & [\varphi_k^\alpha \varphi_j^\beta] + [\varphi_\beta^\alpha \varphi_j^\beta] + \frac{1}{n-r+1} [\theta_j \omega^\alpha] - \frac{1}{n+1} Q_{\beta\alpha j}^{\beta\alpha} [\varphi_k^\sigma \omega^\beta] - \\
& \frac{1}{2} (Q_{\beta j k}^{\beta\alpha} + Q_{\beta k j}^{\beta\alpha}) [\varphi_l^\beta \omega^k] - \frac{n-r+1}{2(n+1)} (R_{\beta\rho k}^{\beta\alpha} [\varphi_j^\rho \omega^k] + R_{\beta\rho j}^{\beta\alpha} [\varphi_k^\rho \omega^k]) + \\
& R_{\beta j \gamma}^\alpha [\omega^\gamma \omega^\beta] + R_{\beta j k}^{\alpha\gamma} [\omega_\gamma^k \omega^\beta] + T_{j k \beta}^\alpha [\omega^\beta \omega^k] + T_{j k l}^\alpha [\omega^l \omega^k] + T_{j k i}^{\alpha\beta} [\omega_\beta^i \omega^k]. \quad (29)
\end{aligned}$$

The conditions

$$T_{j k \beta}^\beta = 0 \quad (30)$$

determine θ_k completely.

From the complete determination of φ_j^α , φ_α , θ_k follows their invariance. We have therefore a set of linearly independent invariant Pfaffian forms whose number is equal to the number of variables. A necessary and sufficient condition for two families of varieties to be equivalent is that a transformation in all our variables exists, which carries one set of Pfaffian forms to the other. Hence the problem of equivalence is solved.

If the given family of varieties is the family of linear spaces, our Pfaffian forms are those which define the infinitesimal transformation between two neighboring projective frames. In the general case they define a projective connection. The actual calculation of the components of the projective connection in terms of the coördinates of the space offers no essential difficulty.

¹ Eisenhart, L. P., *Non-Riemannian Geometry*, New York, 1927.

² Hachtroudi, M., *Les espaces d'éléments à connexion projective normale*, Paris, 1937.

³ We agree that Greek indices run from 1 to r and that Latin indices run from $r+1$ to n .

THE EPIDEMIC CURVE, II

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In a previous note¹ we discussed comparatively the formulations by Soper and by Frost of the progress of an epidemic in the absence of recruitment of susceptibles and came to a relationship between the initial susceptibles S_0 , the residual susceptibles S_E and the number m which on Soper's formulation was the number of susceptibles for a steady state (when there was recruitment). To discuss periodicity Soper made certain approximations which carried his equations over from algebraic form to expressions in terms of the calculus (differential equations). He introduced a variable $u = \log (z/A)$, where z is rate of cases and A is rate of recruits.

This transformation cannot be used if $A = 0$. We wish now to compare the results of Soper's approximation for the case where $A = 0$ with the results we obtained previously.

If τ be the incubation period and $u = \log z$, Soper's differential equations become

$$1 + \tau \frac{du}{dt} = \frac{S}{m} \text{ and } S = m \text{ for } u = \text{maximum.}$$

Then

$$\frac{dS}{dt} = A - z, \text{ and } \frac{d^2u}{dt^2} = \frac{A - e^u}{m}. \quad (1)$$

The first integral is

$$\tau \left(\frac{du}{dt} \right)^2 = \frac{2}{m} (Au - e^u) + \text{const.}$$

and if the constant is determined when $du/dt = 0$ and $u = u_0, t = 0$,

$$t = - \sqrt{\frac{m\tau}{2}} \int_{u_0}^u \frac{du}{\sqrt{A(u - u_0) - (e^u - e^{u_0})}}$$

$$S - m = \int_0^t (A - z)dt = At \pm \sqrt{\frac{m\tau}{2}} \int_{u_0}^u \frac{du}{\sqrt{A(u - u_0) - (e^u - e^{u_0})}},$$

the integral having the negative sign so that $S < m$ when $t > 0$ and the positive sign with $S > m$ when $t < 0$.

Now if $A = 0$, the integrations may be performed in finite form and in terms of z become

$$z = z_m \operatorname{sech}^2 \sqrt{\frac{z_m}{2m\tau}} t$$

$$S = m - \sqrt{2mz_m\tau} \tanh \sqrt{\frac{z_m}{2m\tau}} t.$$

The epidemic curve begins at $t = -\infty$ and runs to $t = +\infty$ instead of being of finite duration, but actually since the number of cases cannot be fractional the curve would be limited for practical purposes to a finite interval of time. The curve of the case rate is symmetrical with respect to its maximum whereas the curves figured stepwise from generation to generation were found to be asymmetrical with the decreasing more abrupt than the increasing portion. This is one difference due to the approximations introduced. Another difference is found in the relation between S_0, S_E and m ; for, setting $t = \mp \infty$,

$$S_0 = m + \sqrt{2mz_m\tau}, S_E = m - \sqrt{2mz_m\tau}$$

and

$$S_0 + S_E = 2m \text{ instead of } \frac{S_E}{S_0} = e^{-\frac{S_0}{m}} \left(1 - \frac{S_E}{S_0}\right).$$

From the second equation we found in tabular form relationships between S_0/m and S_E/S_0 with no restriction on S_0/m . (This was for Frost's formulation as modified by Wilson through the introduction of the law of small numbers; it was impossible to obtain such a relation for Soper's original finite stepwise formulation.) On Soper's formulation in differential equations, we see that since $S_E \geq 0$, $S_0 \leq 2m$ and further that $m \geq 2z_m\tau$.

When therefore we have $A > 0$, as is necessary for periodicity, we cannot expect the stepwise calculations to give the same result as the theory based on Soper's differential equations. For discussing the periodicity ($A > 0$) we will do well to return to Soper's notation, $u = \log(z/A)$. Then

$$t = -\sqrt{\frac{m\tau}{2A}} \int_{u_0}^u \frac{du}{\sqrt{u - u_0 - e^u + e^{u_0}}}.$$

The expression $u - u_0 - e^u + e^{u_0}$ has one root u_0 and another root u_1 (at minimum) which may be written approximately² as

$$-u_1 = C - e^{-(C - e^{-C})}, \quad C = e^{u_0} - u_0.$$

Thus the period is

$$T = \sqrt{\frac{2m\tau}{A}} \int_{u_1}^{u_0} \frac{du}{\sqrt{u - u_0 - e^u + e^{u_0}}}.$$

As the integrand becomes infinite for $u = u_0$ and $u = u_1$ we form the function

$$f(u) = \frac{1}{\sqrt{u - u_0 - e^u + e^{u_0}}} - \frac{1}{\sqrt{(u_0 - u)(e^{u_0} - 1)}} - \frac{1}{\sqrt{(u - u_1)(1 - e^{u_1})}}$$

which remains finite and may be integrated by Simpson's or similar rules, giving³

$$T = \sqrt{\frac{2m\tau}{A}} \left[2\sqrt{u_0 - u_1} \left(\frac{1}{\sqrt{1 - e^{u_1}}} + \frac{1}{\sqrt{e^{u_0} - 1}} \right) + \int_{u_1}^{u_0} f(u) du \right].$$

Soper considers a normal intensity for an epidemic to be where $e^{u_0} = 4$ or $z_m/A = 4$, so that the case rate at maximum is four times the rate of recruitment. In many of our epidemics of measles the ratio z_m/A seems larger than 4. For example in Hedrich's epidemic⁴ of 1930-1931 in

Baltimore z_m/A as estimated (allowance being made for the incompleteness of reporting) was about 10. In Providence⁵ for the 1934-1935 epidemic the maximum number of reported cases in one month was 1953 whereas the recruits were about 350 which gives a ratio of 5.6 but there probably was only about 50% reporting making the true ratio about 11. In Providence in January, 1932, there were 2799 cases reported which gives a ratio of 8.0 and if this be doubled we find 16. A computation of the periods for $e^{u_0} = 4, 6, 10$ shows 7.15, 8.00, 9.75 times $\sqrt{m\tau/A}$. If we take $m\tau/A = 130$ which makes m equal to 5 years of births we find that the periods in the three respective cases would be 3.1, 3.5, 4.3 years.

We can make a stepwise calculation from Soper's finite formulas for the case $S_0 = 36,000$, $A = 500$ per incubation period and $m = 29,000$; the results are:

TIME	CASES	TIME	CASES	TIME	CASES	TIME	CASES
1	500	15	1106	29	62	43	58
2	621	16	882	30	56	44	65
3	768	17	692	31	51	45	74
4	943	18	538	32	47	46	85
5	1144	19	418	33	44	47	99
6	1362	20	326	34	42	48	116
7	1581	21	256	35	41	49	138
8	1776	22	203	36	41	50	166
9	1917	23	163	37	41	51	201
10	1976	24	133	38	42	52	246
11	1936	25	110	39	44	53	303
12	1801	26	93	40	46	54	376
13	1595	27	80	41	49	55	468
14	1352	28	70	42	53		

In this case $e^{u_0} = 3.95$ and $m\tau/A = 58$ so that the computed period would be $7.15\sqrt{58} = 54.5$ incubation periods or a little over two years. However this makes the value of m only a trifle over two years' births.

There is, however, another point in the theory which is worth mentioning. The maximum u_0 less the minimum u_1 is $u_0 - u_1$ and this is the natural logarithm of the ratio of cases at maximum to cases at minimum which for $e^{u_0} = 4, 6, 10$, respectively, gives 3.92, 5.98, 10.00 of which the anti-logarithms are 50, 395, 22,000. The value 50 checks tolerably well with the arithmetical calculation just given. Such a value as 22,000 would of course be impossible except in a very large area because the cases at maximum would have to be 22,000 per incubation period in order for 1 to remain at minimum. In fact the difficulty we have in applying Soper's or Frost's methods to many instances which we actually find in pronounced epidemics is that the cases become fractional and remain so for a long time around minimum—which means that the epidemic would in fact terminate.⁶ To be sure of having one case left in Providence we could not have e^{u_0}

greater than 6, nor greater than 7 in Baltimore; whereas when allowance is made for incomplete reporting the values of e^{u_0} are generally greater than those values.

¹ These PROCEEDINGS, 28, 361-367 (1942).
² The equation $u - u_0 - e^u + e^{u_0} = 0$ may be solved numerically for u_1 but the approximation given is generally sufficiently good.
³ For the case $e^{u_0} = 4$, $u_0 = 1.386$, $u_1 = -2.535$, the total interval is $u_0 - u_1 = 3.921$ and the values of $f(u)$ at intervals of 0.03921 from u_0 to u_1 , inclusive, are -0.53, -0.43, -0.39, -0.37, -0.35, -0.33, -0.32, -0.31, -0.30, -0.29, -0.29. The integral of $f(u)$ by Simpson's Rule is -1.37 and the value of T is 7.15 times $\sqrt{m\tau/A}$.
⁴ *Amer. Jour. Hygiene*, 17, 613-636 (1933).
⁵ See the Report of the Superintendent of Health of the City of Providence for the year 1940. For convenience the table is reproduced below:

MEASLES CASES BY MONTHS IN PROVIDENCE 1917-1940													
YEAR	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	TOTAL
1917	33	47	62	109	119	36	13	7	2	1	8	55	492
1918	55	98	373	1232	1299	780	261	23	8	6	5	3	4143
1919	1	4	4	4	5	4	3	3	1	2	1	3	35
1920	125	127	136	279	404	288	146	38	45	53	190	191	2022
1921	329	585	665	390	266	99	28	10	1	2	7	26	2408
1922	89	4	3	26	25	22	23	19	7	16	131	652	1017
1923	680	1228	1470	687	383	117	29	6	3	10	7	7	4627
1924	5	6	3	11	16	30	15	2	2	1	5	2	98
1925	13	11	6	15	18	30	58	50	13	81	417	1224	1936
1926	2057	1360	648	348	196	105	48	8	1	0	0	4	4775
1927	5	2	1	1	2	2	6	2	0	9	7	23	60
1928	45	112	422	1081	883	800	508	77	18	36	36	61	4079
1929	84	189	261	399	276	111	38	4	3	2	0	0	1367
1930	2	0	1	4	23	46	22	8	1	0	2	0	109
1931	1	2	49	158	456	358	179	99	22	191	337	1648	3400
1932	2799	2037	574	199	81	11	2	0	0	0	0	0	5703
1933	0	0	0	3	3	6	5	2	4	0	1	1	25
1934	4	11	21	18	29	106	44	25	8	5	1	7	279
1935	13	57	343	1351	1953	1279	241	17	4	1	0	48	5307
1936	119	74	92	76	83	17	11	4	0	0	9	77	562
1937	422	811	1184	711	472	129	31	4	0	2	3	3	3772
1938	2	5	4	2	0	0	0	3	1	0	0	3	20
1939	33	35	40	118	317	286	157	64	20	89	267	446	1872
1940	569	495	530	462	543	372	121	20	1	0	1	1	
Total	7485	7300	6890	7684	7852	4934	1989	495	165	507	1435	4385	51221

Epidemics culminate in May, 1918, March, 1921, March, 1923, January, 1926, April, 1928, January, 1932, May, 1935, March, 1937, March (?), 1940. In this period of 262 months there are 9 major peaks, but we must not count both ends. The average time between peaks is 33 ± 7.9 months, not 2 years. For the mean we write 33 ± 2.8 months. In Glasgow we estimate 40 months between peaks from 1888 to 1927, inclusive, based on Soper's data (*J. Roy. Statist. Soc. London*, 93, 34-61 (1929)). How many peaks one counts depends on the interpretation one gives to the qualifying adjective major and what allowance one makes for seasonal interruption of an epidemic.

⁶ In that case measles would recur only when reintroduced after a lapse of time sufficient to build up a sufficient number of susceptibles, the notion of periodicity based on the continuous operation implied in the differential equations or the stepwise calculation would have to be abandoned, and we should properly speak of recurrences rather than of periods.

The difficulty of discussing the ratio of cases at maximum and minimum, and the matter of continuity versus dying out and reintroduction of measles on the basis of reported cases is great. A fairly good estimate of the fraction of total cases which are reported is easy to make, and if it can be assumed that this ratio is more or less constant the arguments here advanced are sound. There are unpublished data for Newton, Mass., collected by Dr. Harold D. Chope for his doctoral thesis (which is partly published in *Virus and Rickettsial Diseases*, Harvard University Press, 1940, pp. 283-308, under the title "A Study of Factors That Influence Reporting of Measles") which throw some doubt on the propriety of assuming a constant ratio.

MEASLES CASES REPORTED AND NOT REPORTED FOR 1936 AND 1934 BY MONTHS

	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.-DEC.	TOTAL
Rep.	18	59	204	295	181	75	4	3	839
Not	14	28	61	66	53	38	36	24	320
%	56	68	67	82	77	66	10	11	72
Rep.	25	59	49	69	55	63	17	4	341
Not	25	36	54	56	31	20	37	40	293
%	50	62	48	60	64	72	32	9	54

The percentage of cases reported differed between the two years, being less in the lighter year and varied seasonally, being less in the lighter months—indeed very low in the summer and autumn. The reporting by years is also interesting:

	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938
Rep.	5	0	71	17	173	63	341	42	839	55	42
Not	59	57	123	120	213	269	293	377	320	64	35
Total	64	57	194	137	386	332	634	419	1159	119	77
%	8	0	35	12	45	19	54	10	72	46	55

The great irregularity between the years (though apparently with an upward trend) in reporting may be noted. The two-year periodicity which might be detected in the reported cases in 1930, 1932, 1934, 1936 largely disappears when one looks at the total cases.

What the annual or seasonal variations in percentages reported in Providence may be we do not know; if the variations were very great they would affect the details of some of the calculations in the text, but apparently they would not destroy the general conclusions. It would improve the statistical discussion of the epidemiology of infectious diseases if there were more detailed studies of the factor of reporting.

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POSSIBLE GROUPS OF AUTOMORPHISMS

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The identity and the group of order 2 are obviously the only groups whose group of automorphisms is the identity since every non-abelian group has a non-cyclic group for its group of inner automorphisms and every abelian group whose order exceeds 2 admits outer proper automorphisms. If a group has the group of order 2 for its group of automorphisms it must be abelian and hence its automorphism of order 2 must result from the automorphism in which every operator corresponds to its inverse since the group of automorphisms of every non-cyclic abelian group is known to be non-abelian.¹ Hence there are three and only three groups which have the group of order 2 for their group of automorphisms, viz., the group of order 3, and the cyclic groups of orders 4 and 6, respectively. The last of these groups is the direct product of two groups and illustrates the elementary theorem that a necessary and sufficient condition that the direct product of two proper groups (groups which differ from the identity) has the same group of automorphisms as one of these groups is that this one involves no subgroup of index 2 and that the other is of order 2.

We proceed to prove that there is no group which has a cyclic group of odd prime power order as its group of automorphisms.² The group of order 3 is obviously the group of lowest order which comes under this theorem and it is the group of lowest order which is not the group of automorphisms of some group. It is well known that the group of automorphisms of the group of prime order p is the cyclic group of order $p - 1$ and this could not be a cyclic group of odd prime power order but it may be a cyclic group whose order is a power of 2. It was noted above that the group of automorphisms of a non-cyclic abelian group is non-abelian and hence such a group could also not have for its group of automorphisms a cyclic group of odd prime power order. Finally, a non-abelian group could not have a cyclic group as its group of automorphisms since its group of inner iso-

morphisms is not cyclic and every subgroup of a cyclic group is itself cyclic. This completes a proof of the theorem that *no group has for its group of automorphisms a cyclic group of odd prime power order and that the only groups which have cyclic groups of order 2^m as their group of automorphisms are those of order $2^m + 1$ whenever this is a prime number.*

If the group of inner automorphisms of a given group G does not coincide with the group of automorphisms of G then the former is an invariant subgroup of the latter. From this well-known theorem it results directly that when a simple group of composite order is the group of automorphisms of G then it is either also the group of inner automorphisms of G and hence it is isomorphic with G with respect to the central of G or G is abelian. In the latter case all of the operators of G besides the identity are of order 2 since the automorphism of an abelian group in which every operator corresponds to its inverse is an invariant operator of order 2 in its group of automorphisms whenever the abelian group contains operators whose order exceeds 2. When it involves no such operators this automorphism is the identity. It therefore results that whenever the group of automorphisms of an abelian group is a simple group of composite order then this abelian group is of order 2^m and of type 1^m , and it is known that every such group has a simple group of composite order for its group of automorphisms whenever $m > 2$.

Suppose that the group of automorphisms of G is a prime power group and that G involves Sylow subgroups whose orders are powers of at least two distinct prime numbers. At least one of these prime numbers cannot divide the order of the group of automorphisms of G and the operators of G whose orders are powers of this prime number must appear in the central of G since otherwise the group of automorphisms of G would not have an order which is a power of a single prime number. Hence it results that when the group of automorphisms of G is a prime power group then all the Sylow subgroups of G except possibly one appear in the central of G and if one of these Sylow subgroups does not appear in the central of G its order is divisible by the prime number which divides the order of the group of automorphisms of G . In all cases G is the direct product of its Sylow subgroups and all the Sylow subgroups of G which appear in its central are of prime order. Hence there results the following theorem: *When the group of automorphisms of a given group G is a prime power group then G is the direct product of its Sylow subgroups and all of these Sylow subgroups except possibly one are of prime order. If G involves a Sylow subgroup which is not of prime order then the order of its group of automorphisms divides the order of the group of automorphisms of G .*

From the theorem just noted it results that when the order of the central of such a G is divisible by an odd prime number then this number must be of the form $2^m + 1$ and the order of the group of automorphisms of G must

be a power of 2 since the group of automorphisms of an abelian group is the direct product of the groups of automorphisms of its Sylow subgroups. The number of such odd prime numbers which may be the orders of groups appearing in the central of G has evidently no upper limit. When such a G contains a Sylow subgroup whose order is divisible by the square of an odd prime number then this subgroup must be non-abelian and the order of G is not divisible by a power of any other prime number except that it may possibly be divisible by 2, but when G contains a Sylow subgroup whose order is a power of 2 then this subgroup need not be non-abelian. As an instance of such a group we may cite the direct product of a cyclic group whose order is a power of 2 and a group of prime orders of the form $2^m + 1$.

One of the most interesting facts relating to possible groups of automorphisms is that the symmetric group of degree n is its own group of automorphisms except when n is either 2 or 6. In the former case the group of automorphisms is the identity, as was noted above, while in the latter case the group of automorphisms has an order which is twice the order of the corresponding symmetric group. It may be of some interest to prove here that whenever the degree of the symmetric group is different from 2 and 6 then the group of automorphisms of the direct product of the symmetric group and the group of order 2 is also its own group of automorphisms. To prove this fact it may first be noted that such a direct product has obviously the corresponding symmetric group as a subgroup of its group of automorphisms. As this group contains one and only one subgroup of index 2 it is possible to obtain an automorphism of the given direct product by letting the operators of the alternating group contained in this symmetric group correspond to themselves while the remaining operators correspond to themselves multiplied by the invariant operator of order 2 in the given direct product.

The automorphism obtained in this way is clearly an invariant automorphism of order 2 under the group of automorphisms. As this group contains two invariant subgroups which have only the identity in common it is the direct product of these two subgroups. A necessary and sufficient condition that the group of inner automorphisms of a group is also its group of automorphisms is that the group admits no outer isomorphisms. Hence the statement on page 152 of the *Survey of Modern Algebra* by Birkhoff and MacLane (1941) that the group of symmetries of the square admits no outer automorphisms implies that the abelian group of inner automorphisms of the octic group is its group of automorphisms. If this were true we would have here a very simple answer to the question on page 233 of Hilton's *Introduction to the Theory of Groups of Finite Order* (1908) which is as follows: Can a non-abelian group have an abelian group of automorphisms? This question was answered in the affirmative by the present writer on page 124 of volume 43 (1914) of the *Messenger of Mathematics*.

It may be added that what is now commonly called the group of automorphisms used to be called group of isomorphisms which is a somewhat shorter but less suggestive term. On the contrary the recently proposed term *byvalue* in place of the much older and more widely used term coefficient seems to be more justifiable because it is both shorter and more suggestive than the term which it aims to replace. See W. Krull *Elementare Algebra*, page 5 (1939). It is evident that automorphisms are very closely related to conjugate operations. The outer automorphisms correspond to conjugate elements under operators which transform a group into itself but do not themselves appear in the group. The concept of conjugate elements is very fundamental in the theory of groups. In fact A. L. Cauchy and some of his followers used the term *system of conjugate substitutions* instead of the term group.

¹ Miller, G. A., *Transactions of the American Mathematical Society*, Vol. 1, p. 395 (1900).

² On page 38, Vol. 10 (1942) of the *Indian Mathematics Student* it is stated incorrectly that "every group can be considered as a group of automorphisms."

FIXED ELEMENTS AND PERIODIC TYPES FOR HOMEOMORPHISMS ON S. L. C. CONTINUA

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We denote by S a semi-locally-connected continuum;¹ the only type of space used. On S only onto-homeomorphisms $T(S) = S$ are considered. This note lends support to the thesis that, since the cyclic element theory² tends to view S as a one-dimensional structure, an orderly approach to the behavior of these onto-homeomorphisms on S can be made through this theory. And that, furthermore, convergence properties³ together with fixed element conditions⁴ may be brought to bear with advantage before the many periodic types for T are distinguished. Reserving the details for publication elsewhere we summarize here a fixed element theorem and some characterizations intended to support these views.

If S is a dendrite (tree) and x is an end-point of S fixed under T then let $p \neq x$ be an end-point and denote the unique arc from x to p by $A(x, p)$. Now $T(p)$ is an end-point and there is a point $q \neq x$ such that $A(x, q) = A(x, p) \cdot A(x, T(p))$. If $T(q) = q$ fails to hold the proper subset relation $A(x, q) \subset T(A(x, q))$ arises from one position for $T(q)$. We ignore the other possibility; it is met by the same argument using the inverse of T . Now

the sum $A(x, q) + T(A(x, q)) + \dots$ of nested arcs has an arc A as its closure and consequently A has two distinct end-points each fixed under T . These methods suitably altered yield the following theorem stated for $T(S) = S$ but with applications in $T(S) \subset S$.

THEOREM (A). *If $N \neq S$ is an invariant node then there exists in S another invariant cyclic element $E \neq N$.*

If B is invariant under T^k , that is, $T^k(B) = B$, it is sometimes said for brevity that the set B is T^k -invariant. If k is the least positive integer giving this equality then the sum of the distinct images of B under iteration of T is the orbit of B and k is the period of B . In the next theorem the orbit of a cut point indicates something of the behavior of T in general. If p is a cut point of S which is not fixed under T then some component R of $S - p$ contains a cyclic element fixed⁴ under T . Hence if $H = \bar{R}$ and $K = S - R$ then H and K are continua such that $S = H + K$, $H \cdot K = p$, and $T(H) \cdot H \neq 0$.

THEOREM (B). *If $T^k(K) \cdot K \neq 0$ and k is the least positive integer giving this intersection then either $T^k(p) = p$, or the T^k -orbit P of p is infinite and the least (T^k -invariant) A -set A which contains P is a cyclic chain having two fixed points as end-points.*

Certain precise consequences can also be stated when this theorem fails to apply. The results so far combine to throw new light on certain properties introduced by Ayres and Whyburn;⁵ among other things these properties are now freed from pointwise almost periodicity requirements.

(a1) If C_1 and C_2 are invariant cyclic elements then every cyclic element in the cyclic chain $C(C_1, C_2)$ is invariant.

(a2) The sum of all invariant cyclic elements of S is a non-empty A -set.

(a3) For every pair of distinct cyclic elements E_1 and E_2 such that $T(E_1) = E_2$, the cyclic chain $C(E_1, E_2)$ contains one and only one invariant cyclic element.

(w1) If X is an invariant A -set in S and $N \neq X$ is an invariant node of X , then there exists a fixed cut point of X .

(w2) Let H and K be any two continua of S such that $S = H + K$ and $p = H \cdot K$ is a cut point of S . If H and K each meets its image under T then $T(p) = p$.

FIRST CHARACTERIZATION THEOREM. *If $T(S) = S$ has one of these five properties it has all of them.*

By assuming that these properties hold for both $T(S) = S$ and $T^2(S) = S$, that is, in general for all powers from T to T^2 , the action of T becomes more clearly defined. We give next a view of this type of situation. If L is the set of all end-points in S and each cyclic element of S in $S - L$ has a finite period then T is said to be *elementwise periodic* on $S - L$. This term is due to Ayres; we introduce a kind of dual concept. If A is an invariant true A -set and each component R of $S - A$ has a finite period

then T is said to be *componentwise periodic* at A ; *componentwise periodic* on S provided this local property holds for every A . We say an A -set A is *bordered* or *unbordered* according as it is possible or not possible to select a point p in A and a true cyclic element E such that $p = A \cdot E$. The interior of a set X is written $\text{Int } X$.

SECOND CHARACTERIZATION THEOREM. *In order that $T(S) = S$ be elementwise periodic on $S - L$ it is necessary and sufficient that one of the following conditions be satisfied:*

(a) *If M is any set in $S - L$ having the property that M contains each cyclic element of S which intersects M then $T(M) \subset M$ implies⁵ $T(M) = M$.*

(b) *T is componentwise periodic, and for every positive integer n , T^n has one of the five properties of Ayres and Whyburn.*

(c) *T is componentwise periodic, and each unbordered T^n -invariant A -set A in S admits a contracting⁷ T^n -approximation which preserves interiority at A —that is, for every A -set C with the property $A \subset \text{Int } C$ there is a third T^n -invariant A -set B such that $A \subset \text{Int } B \subset C$.*

Elementwise periodicity on all of S , or on the complement of the set of all nodes of S , may be characterized directly from the conditions above. The contracting T^n -approximations are directed toward proving the continuity of orbit decompositions; a type which gives rise to interior (open) transformations. Certain methods for establishing the continuity of decompositions by means of these approximations are illustrated using for brevity a strongly restricted space. This gives the essentials of a proof for the theorem⁸ which states that a *period function defined for the points of S relative to $T(S) = S$ and bounded on each cyclic element yields a continuous orbit decomposition*. However, the presence of disjoint closed invariant sets rather than point-orbits in the illustration points the way to generalizations in this direction.

In the absence of any statement to the contrary page numbers refer to the Colloquium Publication, *Analytic Topology*, by G. T. Whyburn, New York, 1942. See also the references in this book.

¹ P. 19.

² Pp. 64-98 for terminology and results used freely below.

³ P. 69.

⁴ P. 242. Theorem of Ayres. *Fund. Math.*, 16, 332 (1932).

⁵ Pp. 247-249.

⁶ Compare Theorem (1.2), p. 240.

⁷ For an "expanding" approximation, see Theorem (4.7), p. 249. Omitting the p. a. p. assumption this too is a characterization.

⁸ Abstract in *Bull. Am. Math. Soc.*, 45, 82 (1939). Also see Theorem (5.1), p. 251 and Theorem (6.42), p. 258.

ATROPINESTERASE, A GENETICALLY DETERMINED ENZYME IN THE RABBIT

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Introduction.—Hereditary differences in enzyme activity are well known among both plants and animals, particularly in so far as they have to do with irregularities in pigment formation.^{10, 18} In animals such differences are not restricted to pigment formation but have to do with many other physiological activities such as the xanthophyllase activity which determines the presence or absence of yellow color of body fat in the rabbit,^{1, 11} the amylase activity in the digestive juices of silkworm larvae⁷ and the uricase activity which determines the amount of nitrogen excreted as uric acid in the dog.⁶ In man alkaptonuria, steatorrhea, hematoporphyrria, pentosuria and cystinuria are other biochemical differences which are suspected to have a similar background.

Recently interest has been revived in the observation, first reported by Fleischmann³ and subsequently confirmed by others in various parts of the world, that the blood of certain rabbits can destroy atropine while that of others cannot. Studies of some properties of the enzyme in the serums of those rabbits capable of hydrolyzing atropine have been carried out by Glick,⁴ and Glick and Glaubach⁵ have investigated the distribution of the atropinesterase among certain tissues in these animals. The possibility that atropinesterase in rabbit serum is an inherited factor was recognized by Levy and Michel⁷ but no supporting data have been forthcoming. The present coöperative investigation is an attempt to determine the genetic properties of the enzyme.

Methods.—Blood samples of sufficient volume to provide 0.5 cc. of serum have been obtained from animals at three months of age or older of the proper matings in the laboratory at Brown, centrifuged to remove the cells and placed in the mail for examination within three days at Newark. The enzyme activity was determined by the manometric method employing the Warburg apparatus in the manner previously described.^{4, 5} The activity was expressed as atropinesterase units per 100 mg. of serum. The unit is defined as the amount of enzyme required to liberate 1 c. mm. CO₂ in 300 minutes at 30° in a total volume of 4 ml. in the bicarbonate-Ringer medium containing a concentration of substrate (0.25% atropine sulphate) sufficient to achieve the maximum rate of hydrolysis.

Data and Discussion.—Preliminary examination of five unrelated families showed that about 55 per cent of the 181 rabbits examined possessed the

atropinesterase. This is a considerably higher proportion than previously obtained by other investigators and suggests the existence of racial differences, a fact that becomes more apparent when this population is separated into its constituent parts. Two of the families—III, a New Zealand White race which has been closely bred for more than 10 generations, and the *A* race of Castle, a small-sized multiple-recessive strain—appear to lack the enzyme entirely. The other three families possess it in a high proportion of the individuals. Family V, a pure chinchilla race, also closely bred for 12 generations, and family X, a more heterogeneous race originated by hybridization of the small race with other genetic stocks, produce both enzyme-possessing and non-enzyme-possessing offspring. In the first family, among 50 individuals tested the proportion was equal; in the second, 75 per cent of 87 animals examined were atropinesterase-producing. Only 14 individuals have been examined in family IIc, of which three did not possess the enzyme. Of these families, III, V and X at least, show significant genetic differences. No entire family was found which does not produce some individuals lacking the enzyme.

The evidence as to the hereditary nature of these differences is as follows. In a total of 69 offspring obtained from parents lacking the enzyme, none have shown any trace of the enzyme. Parents possessing the enzyme, on the other hand, may or may not transmit the character to their offspring. If those which do possess it are paired with mates which do not, one of two alternatives follows. Either the offspring *all* possess the enzyme or they are about equally divided between those which possess it and those which do not. This indicates homozygosity in the first case and heterozygosity in the second case, of the parent possessing the character. Thirty-seven individuals have been obtained from the first type of mating, all of which were positive. In a population of 173 individuals obtained from the second type of mating, 88 possessed the enzyme in their blood and 85 did not, which is a close approximation to the equality expected from a monohybrid backcross. From known hybrids mated with each other, 68 young possessed the enzyme and 30 lacked it. This departure from the expected 3:1 ratio is not significant since it is less than twice the probable error.

From these results it appears that the ability to produce the enzyme is dominant over its absence. Dominance is probably not complete, however, since animals known to be heterozygous show a lower mean value of enzyme production than those which are homozygous. For 25 animals known to be heterozygous the mean value was 107 with a range of 52–174. For 4 animals known to be homozygous the mean value was 271 with a range of 232–348.

It is also interesting to note that the enzyme is not present at birth but first manifests itself at one to two months of age. Forty-five animals have been bled from the heart at birth and at monthly intervals two or three

times thereafter. Thirty-nine of these possessed at least one homozygous parent and hence could be expected to have the enzyme in their blood at three months of age. Six were from a heterozygous male and a non-enzyme mother. None of those examined at birth had demonstrable activity in their blood. Twelve out of 16 of the individuals of the first group, examined at the end of the first month, possessed the enzyme in substantial amounts and two others had a trace. All of them were active at subsequent examinations. Of the second group none possessed the enzyme at the end of the first month but one of them did show it at later examinations.

Similar quantitative differences and also differences in the age of production of the enzyme are apparent in the amylase of the digestive juice of the silkworm,⁷ and in several enzymes of the pig.⁸

Through information supplied by Dr. C. I. Wright of the National Institute of Health, Bethesda, Md., who had previously discovered the enzy-

TABLE 1

EVIDENCE FOR GENETIC LINKAGE BETWEEN GENES *As* AND *E*
Backcross progeny from F^1 double heterozygote \times double recessive

	NON-CROSSOVERS		CROSSOVERS		POPULATION
	$\frac{E\ As}{10}$	$\frac{e\ as}{12}$	$\frac{E\ as}{4}$	$\frac{e\ As}{4}$	
Coupling					30
Repulsion	$\frac{E\ as}{4}$	$\frac{e\ As}{5}$	$\frac{E\ As}{1}$	$\frac{e\ as}{2}$	12
Totals	31		11		42

The deviation from equality, 10, is 4.5 times the P.E., 2.19, and so clearly significant. The indicated crossover percentage is 26.2 ± 5.2 .

matic hydrolysis of certain morphine derivatives,¹² there is reason to believe that the enzyme which hydrolyzes atropine may be identical with that which hydrolyzes monoacetylmorphine. Nineteen serums from our laboratory and 8 from his own have been examined by Dr. Wright. Each sample was examined for the hydrolysis of both substrates under the same conditions. All of the 17 individual bloods which hydrolyzed the one compound also hydrolyzed the other, whereas those of 10 individuals hydrolyzed neither. According to Dr. Wright, the order of activity is the same when the serums are arranged as to enzyme concentration for either "monoacetylmorphinease" or "atropinesterase." The initial rate of hydrolysis of the former is somewhat greater than for the latter but the time required for complete hydrolysis is approximately the same, due to the difference in the order of the two reactions.

For the gene responsible for atropinesterase production we shall use the symbol *As* and for its recessive allele, *as*. This gene apparently is borne

on the same chromosome of the rabbit as is the gene *E* for the extension of black pigment in the coat, for in matings between a double recessive individual and an F_1 double heterozygote, crossover recombinations are significantly fewer than non-crossovers, the two classes being 11 and 31, respectively (table 1), whereas equality would be expected if no linkage existed. Tentatively the genes *As* and *E* are regarded as members of a sixth linkage group of the rabbit. Segregation of both of these pairs of genes in this population is entirely regular when they are considered separately.

We regret that due to the national emergency, which has made it impossible for one of us to continue the work, a more accurate determination of the actual strength of this linkage association cannot be obtained at this time. In comparison, however, similar data have been obtained from crosses involving combinations of *As* and the agouti gene *A* and the color gene *C*. Nine and 8 offspring, respectively, have been obtained from these matings and the offspring are as equally divided between crossover and non-crossover combinations as possible.

Considering the population as a whole there is an obvious tendency for females to manifest greater enzyme activity than males, although no significant difference in the distribution of the character to the two sexes is manifest. Close scrutiny of the individual matings, however, reveals that in the backcross matings (*Asas* \times *asas*) this tendency is quite pronounced, the average male and female titers being 98 and 129, respectively, and there is a statistically significant tendency for females to possess the character more often than males. A similar situation occurs in the F_2 although less pronounced. The full meaning of this observation is not apparent. It seems probable, however, that possession of atropinesterase is not in itself a sex-linked character since in the same backcross population sufficient numbers have been obtained to indicate that it makes no difference whether the *As* gene is derived from the mother or the father. It seems more probable that this peculiarity is the result of a difference in the genetic milieu of the two sexes, perhaps acting secondarily through the medium of the sex hormones. It is interesting to compare these results with the similar behavior of cholinesterase in rats and mice in which age and also sex are important factors.²

Conclusion.—Rabbits which have in their blood serum an enzyme capable of hydrolyzing atropine (and monoacetylmorphine) inherit that peculiarity in a gene (*As*) borne in the same chromosome as the gene (*E*) for the extension of black pigment in the coat. The gene (*As*) is incompletely dominant, homozygotes producing the enzyme more effectively than heterozygotes. The enzyme is not present at birth but appears first at about one month of age, and tends to occur in greater concentration in females and to be demonstrable in a higher percentage of them than in males.

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NOTE ON THE TIME-INTENSITY FACTOR IN RADIOBIOLOGY

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In many radiobiological reactions the effect of a given dosage of radiation is found to depend on the "time-intensity factor," that is, to be a direct function of the intensity ("intensity effect") and to be lower when the treatment is intermittent than when it is continuous, the intensity remaining the same ("fractionation effect"). This phenomenon has been generally attributed to recovery of the biological material from the action of the radiation. The intensity effects have been determined by comparing the results of continuous irradiation with constant dose and different intensities. They have been the object of most experimental and theoretical investigations in this field, although they do not seem to supply, *per se*, crucial information on the course of recovery. In a recent study of the intensity effect on x-ray induced chromosomal aberrations in *Tradescantia* microspores,¹ one of us (L. D. M.) came to the conclusion, to be amplified and generalized in this note, that fractionation experiments, consisting of two high-intensity irradiations separated by a variable intermission, should be better suited to the investigation of the time-intensity factor. This is because in fractionation experiments radiation can usually be delivered in a short time, during which recovery is negligible, and recovery

may be allowed to take place mainly during the intermission. The application of more than two separate irradiations seems to constitute an unnecessary complication. The basis for this conclusion was an analysis of the assumed "two-hit" mechanism of production of chromosomal rearrangements.

It has been postulated by Sax² that chromosomal breaks are produced by x-rays in *Tradescantia* microspores at a rate proportional to the dosage, and that an observable rearrangement occurs when two or more breaks are available at the same time in the same microspore. The dependence on the time-intensity factor has been attributed to recovery (or "restitution") of single breaks which otherwise would have contributed to the formation of rearrangements. Since previous experiments had not succeeded in yielding definite information on the process of restitution, the following experimental design was suggested as the most efficient. Microspores should be subjected to an x-ray dose D_1 , followed by an intermission of duration t and then by a second dose D_2 , each dose being delivered within a short time, during which restitution of single breaks is negligible.¹ In the same study a mathematical formula was derived that permits one to gather substantial information from data accessible through an experiment of this type. The derivation of this formula was based, however, on very special assumptions, and the wider scope* of its application was not immediately realized. It is the purpose of this note to derive first an analogous formula starting from less special assumptions, and then to proceed to discuss how the same formula, as well as related ones, can be applied to a broader class of phenomena.

I. The fraction of microspores, $f(D_1)$, in which an observable rearrangement is induced by a single dosage D_1 , delivered within a sufficiently short time, is a function of the dosage only, and can be determined experimentally. The fraction of microspores in which an observable rearrangement is induced by a fractionated treatment such as that described above is a function of both dosages and of the intermission, $F(D_1, t, D_2)$, that can also be determined experimentally. We shall discuss the dependence of F on the various factors involved, on the basis of the previously postulated mechanism.

After the first dose there will be a fraction $f(D_1)$ of the microspores in which two or more breaks have been produced within a short time so that a rearrangement will certainly result. There will also be another fraction, which we indicate by $g(D_1)$, wherein only one break has been produced. During the intermission, some of the single breaks will undergo restitution. By the time the second dose is given, only a fraction of the initial number of single breaks is left; if this fraction depends on the duration t of the intermission we may indicate it by $r(t)$. Experimental determination of this fraction $r(t)$ is the actual object of the investigation considered here.

At the start of the second irradiation there will be three groups of microspores: (1) a fraction $f(D_1)$ which will show rearrangements; (2) a fraction $g(D_1)r(t)$ in which one single break is still present, and (3) the remaining fraction $1 - f(D_1) - g(D_1)r(t)$ in which no break exists. The effect of the second dose D_2 on this complex population can then be computed as follows. No effect of the first dose is present in group (3), so that a completely new rearrangement requiring two or more breaks will be produced in a fraction $f(D_2)$ of it. In group (2) it is sufficient to produce one or more new breaks to have a rearrangement: The second dose (D_2) will produce in this group one break in a fraction $g(D_2)$ and two or more breaks in a fraction $f(D_2)$. The total fraction of rearrangements out of group (2) is, therefore, $g(D_2) + f(D_2)$. The entire group (1) will be scored anyway as showing a rearrangement, so that it will not show any apparent effect from the second dose. The final scoring of rearrangements will thus include a fraction $f(D_1)$ of all microspores, contributed by group (1), a fraction $g(D_1)r(t)[g(D_2) + f(D_2)]$ contributed by group (2), a fraction $[1 - f(D_1) - g(D_1)r(t)] f(D_2)$ contributed by group (3). Therefore the total effect of the fractional treatment on the whole microspore population can be written as:

$$\begin{aligned} F(D_1, t, D_2) &= f(D_1) + g(D_1)r(t)[g(D_2) + f(D_2)] + \\ &\quad [1 - f(D_1) - g(D_1)r(t)] f(D_2) \\ &= f(D_1) + f(D_2) - f(D_1)f(D_2) + g(D_1)r(t)g(D_2) \end{aligned} \quad (1)$$

It is seen now that this quantity $F(D_1, t, D_2)$ consists of two parts. One part, that we call

$$\varphi(D_1, D_2) = f(D_1) + f(D_2) - f(D_1)f(D_2) \quad (2)$$

depends only on the dosages D_1 and D_2 and on the experimentally determinable function f . The other part $g(D_1)r(t)g(D_2)$ is proportional to the unknown function $r(t)$ through the unknown quantities $g(D_1)$, $g(D_2)$. On account of the definition of $r(t)$, $r(0) = 1$; the function $r(t)$ can now be calculated in terms of *experimentally determinable quantities*:

$$r(t) = \frac{r(t)}{r(0)} = \frac{g(D_1)r(t)g(D_2)}{g(D_1)r(0)g(D_2)} = \frac{F(D_1, t, D_2) - \varphi(D_1, D_2)}{F(D_1, 0, D_2) - \varphi(D_1, D_2)} \quad (3)$$

The analogous formula previously derived¹ was based on Swann-Del Rosario's assumption² that $r(t) = e^{-\lambda t}$. This new formula does not make use of any such assumption, yet it serves to test directly not only whether $r(t) = e^{-\lambda t}$ but also whether $r(t) = 1$ for $t < \tau$, $r(t) = 0$ for $t > \tau$ (τ being the lifetime of a chromosome break), as suggested by Lea⁴, or is a still different function.

II. In the case of complete restitution of single breaks which may, but

need not, occur when t is very large, we should have $r(t_{\infty}) = 0$, and hence $F(D_1, t_{\infty}, D_2) = \varphi(D_1, D_2)$. This indicates that the quantity $\varphi(D_1, D_2)$ defined by formula (2) is the total effect of a fractionated treatment under the specific condition that at the start of the second dose all microspores carry either a rearrangement or no break at all.

Whenever the total effect F of a fractionation experiment exceeds φ , it is because at the start of the second dose some microspores carry one single break, that is, show an intermediate, not directly observable, effect of the first dose. This effect, however, becomes experimentally detectable as a sort of "sensitization," because the effect of the second dose on these broken microspores is greater than it would be on microspores carrying no break. The difference between F and φ may then be taken as a new function S measuring the effect of sensitization:

$$S(D_1, t, D_2) = F(D_1, t, D_2) - \varphi(D_1, D_2) = F(D_1, t, D_2) - f(D_1) - f(D_2) + f(D_1)f(D_2) \quad (4)$$

(the latter equality derives from (2)). Comparing (4) with (1) it follows that:

$$S(D_1, t, D_2) = g(D_1)r(t)g(D_2). \quad (4')$$

Furthermore, comparing (4) or (4') with (3), it follows that (3) can be written as:

$$r(t) = \frac{S(D_1, t, D_2)}{S(D_1, 0, D_2)} \quad (3')$$

showing that the recovery function $r(t)$ measures the decline of the sensitization effect S .

It is recovery from this partial, not directly observable, effect of sensitization that we are dealing with, and not recovery from the final observable effect. The latter may be either non-existent (according to the nature of the biological material or the criterion chosen as effect) or may be studied and evaluated directly.⁵

III. It is possible to extend this discussion to a broader class of radiobiologic phenomena, whose mechanism need not be similar to the one postulated for *Tradescantia* microspores. In fact the definition and the experimental determination of the observable effects indicated by $F(D_1, t, D_2)$ and $f(D_1)$ or $f(D_2)$ are perfectly general and in no way dependent on the particular mechanism that is postulated. Whenever a biological effect of radiation is measured as the frequency of occurrence of an observable event, in sufficiently stable biological material, it should be possible to determine the effect of sensitization S experimentally, through formula (4). For a graphical representation of the quantity S in the special case of $t = 0$, see figure 1.

The interpretation of this quantity remains the same as given above, with exceptions to be considered later. The decline of the effect of sensitization S , as the intermission t increases, may be taken as an index of recovery. This index is identical with $r(t)$ (formula (3')) when the theory developed above for *Tradescantia* can be applied. In general, however, it may depend also on D_1 and D_2 because: (1) the course of recovery may depend on the degree of sensitization achieved by the first irradiation with dose D_1 ; and (2) the nature of the observed results may be such that the effect of sensitization as determined by this method may depend on the magnitude of D_2 . Accordingly one may replace $r(t)$ by a general index:

$$R = \frac{S(D_1, t, D_2)}{S(D_1, 0, D_2)} \quad (3'')$$

which can be studied under different conditions.

The experimental determination of the effect of sensitization S for different values of D_1 , t and D_2 permits us to reverse the procedure developed for *Tradescantia* and to test whether or not the mechanism of sensitization and recovery has the same characteristics as the one postulated for *Tradescantia*. Various analytical or graphical procedures may serve to determine whether or not a function of several variables, such as S in formula (4'), is actually the product of several functions, each of them depending on a single variable. First, the index R may be plotted as a function of t for a number of different values of D_1 and D_2 , and it may be verified whether or not all curves so obtained coincide; if they do, R is a function of the interval t only, which may be called again $r(t)$, and S is then the product of its own value at $t = 0$ and of this separate function $r(t)$, namely, $S(D_1, t, D_2) = S(D_1, 0, D_2)r(t)$. The next step is to check whether $S(D_1, 0, D_2)$ itself is the product of two separate functions $g(D_1)$ and $g(D_2)$.

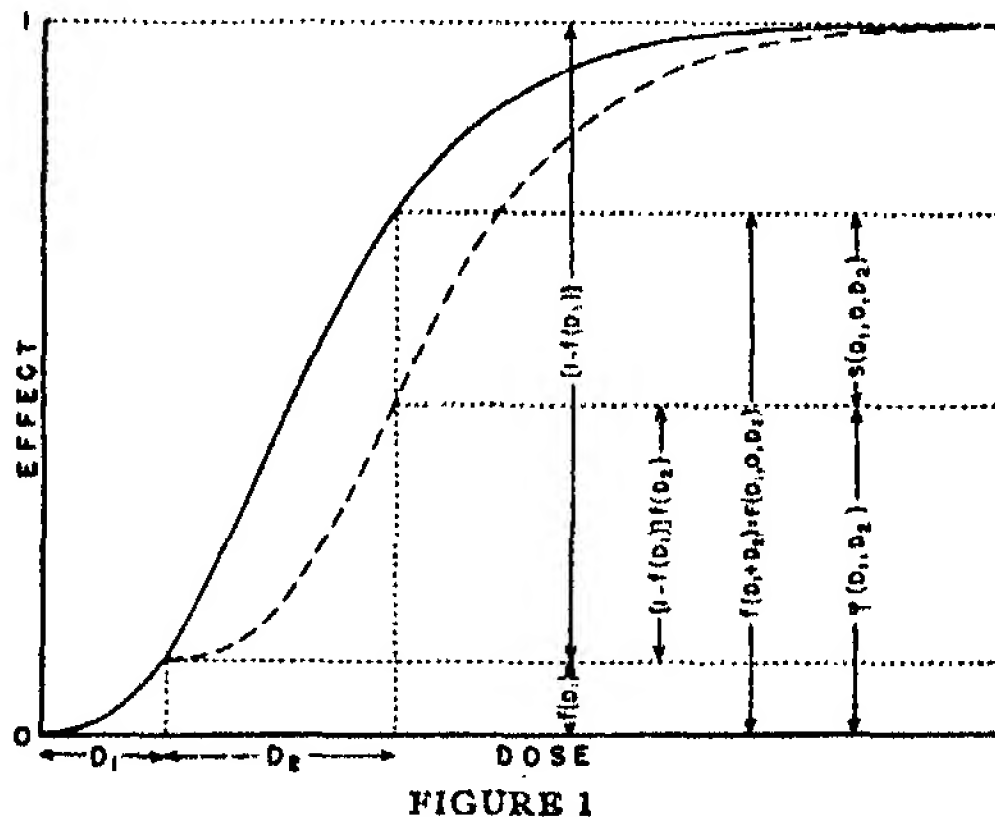


FIGURE 1
Graphical representation of the effect of sensitization as derived from dose-effect relationships.

Dose-effect curve $f(D_1)$ for a single high-intensity irradiation, ———.

Same curve in a different scale $(1 - f(D_1) : 1)$ referring to a second irradiation, - - - - -.

When there is an intermission, the total effect $F(D_1, t, D_2)$ falls short of $f(D_1 + D_2)$ and consequently the effect of sensitization $S(D_1, t, D_2)$ falls short of $S(D_1, 0, D_2)$.

When $t = 0$, the two doses are equivalent to a single dose $D_1 + D_2$; therefore:

$$F(D_1, 0, D_2) = f(D_1 + D_2)$$

and

$$S(D_1, 0, D_2) = f(D_1 + D_2) - f(D_1) - f(D_2) + f(D_1)f(D_2).$$

Thus $S(D_1, 0, D_2)$ depends only on the dose-effect curve for short irradiations and is symmetrical with respect to D_1 and D_2 . The quantity $S(D_1, 0, D_2)$ may now be plotted as a function of D_1 for a number of different values of D_2 , and it may be checked whether or not all curves so obtained differ only by a change of scale in the ordinates. If they do, $S(D_1, 0, D_2)$ is actually the product of two separate functions $g(D_1)$ and $g(D_2)$, and also the curve representing $g(D_1)$ itself differs from any one of these curves only by a proper choice of the scale in the ordinates (such as to fulfill the equation $S(D_1, 0, D_1) = g^2(D_1)$).

The theory for *Tradescantia* leading to formula (4') was derived on the assumption that the production of chromosomal rearrangements takes place according to a "two-hit" mechanism. Conversely, it can be proved mathematically⁶ that the function $S(D_1, 0, D_2)$ is a product of $g(D_1)$ and $g(D_2)$ when, and only when, the dose-action curve $f(D_1)$ fulfills conditions slightly more general than those postulated for a "two-hit curve" by Blau and Altenburger.⁷

When the effect of sensitization S cannot be represented by formula (4'), it may be possible to postulate a more complex mechanism of sensitization and recovery and to describe its mathematical consequences by the methods used above for *Tradescantia*. The effect of sensitization S should then be represented by a sum of terms, each of which has the form indicated in (4'). Experiments aimed at gathering information about the mechanism of sensitization and recovery should therefore determine first the function $S(D_1, t, D_2)$ and next try to break it up into such a sum of terms.

IV. Cases must be considered, in which the function S can still be defined and determined experimentally through formula (4'), but its interpretation as an effect of "sensitization" is not straightforward. For instance, there might be some secondary effect of radiation resulting in partial inhibition of—or resistance to—the main observable effect. This secondary effect, although started by the first dose, could become increasingly intense as subsequent phenomena develop during the intermission, so as to act by itself as a time-intensity factor. In such a case the function S would not measure the "sensitization" proper, but the difference between the sensitization effect and the opposite "inhibition" effect. If the latter effect becomes more important than the former, the function S

becomes negative, which would not be expected to occur on the basis of our earlier considerations.

Another difficulty arises from the possibility that the treated material is initially variable in its sensitivity to radiation. If so, the first dose would induce a final, observable, effect preferentially in the most sensitive organisms, while the second dose would act on a population already selected for higher resistance to radiation. This situation results in an apparent reduction of the effect of sensitization. An effect of this type is important whenever the variability of the material significantly affects the shape of the dose-effect curve for short treatments. This might be the case in experiments like those of MacComb and Quimby,⁸ on the production of erythema in human skin: the percentage of patients showing pigmentation plotted against the dosage of a short treatment yields a threshold-type curve, in which, however, the threshold is not ideally sharp. It seems a reasonable interpretation that the threshold would actually be very sharp except for individual variability. In the discussion of the time-intensity factor, one may try to eliminate the effect of variability, by estimating what the experimental results would be in absence of variability. This is frequently done in the case of threshold-type curves, by discussing the ideal case of a perfectly sharp threshold instead of the observed one. When the threshold is ideally sharp, the observable quantities, i.e., $f(D_1)$, $f(D_2)$, $F(D_1, t, D_2)$, can be only zero or unity, and hence the same holds for the derived quantities $\varphi(D_1, D_2)$ and $S(D_1, t, D_2)$. The purpose of experimental investigation should then be to determine all the possible ways of giving a threshold treatment. Each threshold treatment consists of a set of values D_1, t, D_2 lying on the boundary at which the effect of sensitization jumps from zero to one. Such an investigation was actually carried out by MacComb and Quimby, except that these authors kept the two doses equal ($D_1 = D_2$) and thus did not determine the whole range of threshold treatments but only a cross-section of it. For instance, in order to test Lea's theory⁹ on the elimination of poisonous substances for the case of skin erythema, D_1 should rather be kept constant, and then it may be determined how large the second dose D_2 must be, as a function of the duration t of the intermission, in order to achieve a threshold reaction.

Summary.—X-ray treatments consisting of two irradiations, delivered within a sufficiently short time and separated by variable time intervals, seem to be most suitable for the investigation of those recovery processes that express themselves through the time-intensity factor. From experimental data thus obtained, an index of recovery R can be calculated, which should prove useful in studying the course of recovery. Experimental tests are suggested for determining the complexity of the process. The discussion leads to a more comprehensive point of view with reference to various radiobiological theories previously proposed.

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SOME INTERRELATIONSHIPS OF PYRIDOXINE, ALANINE AND GLYCINE IN THEIR EFFECT ON CERTAIN LACTIC ACID BACTERIA

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Various investigators^{1, 2, 3} have shown that pyridoxine (vitamin B₆) under certain conditions is a necessary growth factor for certain species of lactic acid bacteria. One such organism is *Streptococcus lactis* R. In a detailed study of the requirement of this organism for pyridoxine, Snell, *et al.*,⁴ showed that there existed in natural products a substance which could be derived from pyridoxine by metabolic processes, which far surpasses pyridoxine in its physiological activity for this organism. To distinguish this substance from synthetic pyridoxine it was tentatively called "pseudopyridoxine." It was later shown⁵ that if heat sterilization were avoided, pyridoxine was almost without growth-promoting activity for this organism. It became highly active on autoclaving with the basal culture medium. The degree of activity increased as the period of heating was increased. The effect was traced to interaction during autoclaving between pyridoxine and the amino acids of the medium; presumably "pseudopyridoxine" or a substance of similarly high activity for the test organism was formed in small amounts.

While investigating the effect of individual amino acids in promoting the above "activation" of pyridoxine during heating, it was found that large amounts of alanine completely replaced pyridoxine as a growth factor for this organism. Glycine, on the other hand, inhibited growth. This inhibitory effect of glycine was removed, within limits, by additional amounts of pyridoxine, but not by additional amounts of any of the other vitamins. It was similarly removed—even more effectively—by alanine,

but not by other amino acids. Details of these findings are presented below.

Experimental.—The basal medium used, and the conditions of testing, were exactly similar to those previously described.⁴ The test organism was in all cases *Streptococcus lactis* R. Table 1 shows the comparative effects of pyridoxine, *dl*-alanine and glycine on growth. The maximum growth effect of pyridoxine is reached under these conditions at a concentration of 3 γ per 10 cc. of medium. *dl*-Alanine promotes growth to the same level when 1 to 3 mg. is added per 10 cc. This comparatively low activity of alanine suggests that its activity might be due to contamination with a substance possessing pyridoxine activity. That this is not the case is shown by the following considerations. Synthetic *dl*-alanine obtained from three different sources had exactly the same activity. This activity was unchanged after solution in water, treatment with an equal weight of activated charcoal, evaporation and recrystallization. Finally, the activity of *dl*-alanine was destroyed by nitrous acid treatment; pyridoxine is unaffected by such treatment.⁶ Separate experiments showed that *dl*-alanine would support continued heavy growth of the organism through repeated subcultures in the complete absence of pyridoxine. Under the conditions described, therefore, alanine in high concentration completely replaces pyridoxine in its growth effect on this organism.

TABLE 1

THE EFFECT OF PYRIDOXINE, ALANINE AND GLYCINE ON GROWTH OF *Streptococcus lactis* R.

SUBSTANCE TESTED	AMOUNT ADDED γ PER 10 CC.	GALVANOMETER READING*
.....	20
Pyridoxine hydrochloride	0.2	30
	0.5	44
	1.0	55
	3.0	68
	10.0	68
<i>dl</i> -Alanine	100	21
	300	32
	500	53
	1000	68
	3000	73
Glycine	1000	8.8
	5000	2.8

* Distilled water reads zero; a reading of 100 is complete opacity.

Addition of glycine to the basal medium suppressed growth below that which occurred in the control tubes. Slight growth in such unsupplemented tubes occurs because of carry-over with the inoculum of small amounts of

substances which have pyridoxine activity, or the incomplete removal of such substances from the hydrolyzed casein of the basal medium. When extreme care is taken in preparing the basal medium, and small, washed inocula are used, no growth occurs in the unsupplemented medium, and addition of glycine to such a medium results in no apparent change. The inhibitory effect of glycine is more clearly seen when maximum growth of the organism is permitted by the addition of pyridoxine or of alanine. Results of such an experiment are shown in table 2. From 1 γ to 3 γ of pyridoxine hydrochloride was sufficient to permit growth to proceed to a maximum. No greater growth was attained when as much as 300 γ of the vitamin was added. In the presence of only 1 γ of the vitamin addition of 2 mg. of glycine suppressed growth to approximately the level reached in the control tubes. As the amount of pyridoxine is increased, the amount of glycine required to suppress growth to the level of the control tubes increases: thus with 3 γ pyridoxine hydrochloride, 5 mg. of glycine is required; with 10 γ pyridoxine nearly 10 mg. of glycine is required; with 30 γ of pyridoxine, 30 mg. of glycine is required for inhibition. The inhibitory effect of glycine is thus overcome to a very considerable extent by the presence of pyridoxine in large amounts. More than 30 mg. of glycine is apparently irreversibly toxic for this organism under these conditions. Just as alanine replaces pyridoxine in its growth effect for this organism, so it

TABLE 2
INHIBITORY EFFECT OF GLYCINE ON GROWTH OF *Streptococcus lactis* AND ITS REVERSAL
BY PYRIDOXINE AND ALANINE

ADDITIONS TO CULTURE MEDIUM			ADDITIONS TO CULTURE MEDIUM		
GLYCINE MG. PER 10 CC.	PYRIDOXINE HYDROCHLOR- IDE, γ PER 10 CC.	GALVANO- METER READING*	GLYCINE, MG. PER 10 CC.	DL-ALANINE, MG. PER 10 CC.	GALVANO- METER READING*
0	0	19	0	0	16
0	1	58	0	1	69
0	3	64	0	3	73
0	10	63	0	10	73
0	30	60	0	30	73
0	300	58	1	1	65
1	1	36	3	1	41
2	1	21	10	1	10
2	3	50	1	10	73
5	3	21	3	10	72
10	3	3	10	10	71
2	10	60	30	10	66
5	10	43	100	10	12
10	10	15	100	30	12
5	30	58
10	30	38
30	30	18
30	300	14

* As in table 1.

also reverses the inhibitory effect of glycine. In this experiment, 1 to 3 mg. of alanine permitted maximum growth to occur. With 1 mg. of *dl*-alanine present, about 10 mg. of glycine was required to reduce growth to a level below that reached in the control tube. With 10 mg. of *dl*-alanine, 100 mg. of glycine was required to produce the same effect. Although pyridoxine on the weight basis is much more active than alanine in effecting reversal of glycine inhibition, alanine reverses this inhibition when the glycine concentration is higher. When 100 mg. of glycine is used, the inhibition is not reversed by additional amounts of alanine.

The specificity of these responses is shown in table 3. Of the water-soluble vitamins tested, only pyridoxine was effective in promoting growth on this medium and reversing growth inhibition produced by glycine. Similarly, each of the amino acids so far obtained from casein hydrolyzates was tested. Only those fairly closely related to alanine are listed in the table. Of these, only alanine was effective in duplicating the growth effect of pyridoxine, and in reversing growth inhibition produced by glycine. Each of the other amino acids was completely ineffective in this respect. Serine and threonine show slight inhibitory effects in the same direction as does glycine.

The effectiveness of various compounds related in structure to glycine in producing inhibition of growth was further tested. These results are given in table 4. β -Alanine, *dl*-serine and *dl*-threonine inhibited growth, but their effectiveness was considerably less than that of glycine. In each case the growth inhibition was prevented by simultaneous addition to the medium of additional amounts of pyridoxine or of alanine. As with glycine (table 2), this inhibition was reversible only within a limited range of concentrations, above which inhibition of growth by these compounds was not reversed by additional amounts of pyridoxine or of alanine.

The fact that added alanine replaces pyridoxine for *S. lactis*, but growth does not occur on the basal medium without pyridoxine or alanine, suggests that this organism is incapable of synthesizing alanine. This seemed rather unusual, since lactic acid is the chief product produced by the organism during growth. The amino acid requirements of the test organism were therefore determined. The hydrolyzed casein of the basal medium was replaced with a mixture of all of the amino acids listed as occurring in casein⁷ with the exception of β -hydroxyglutamic acid. The neutralized mixture was added to the medium at such a level that each 10 cc. of medium contained 1 mg. of each amino acid. Ten milligrams of glutamic acid and 3 γ of pyridoxine hydrochloride were added per tube. The effect of omitting individual amino acids from the mixture was then determined. Results are shown in table 5. Alanine, glycine, threonine and serine are among those amino acids which are essential for the growth of this organism. While alanine in high concentration replaces pyridoxine, the addition of excess

TABLE 3
SPECIFICITY OF PYRIDOXINE AND *dl*-ALANINE IN PROMOTING GROWTH AND REVERSING
INHIBITION OF GROWTH BY GLYCINE

SUBSTANCE TESTED	AMOUNT ADDED, γ PER 10 CC.	UNSUPPLE- MENTED	GALVANOMETER READING*	
			3 γ PYRIDOXINE HYDROCHLORIDE AND 4 MG. GLY- CINE ADDED PER 10 CC.	
.....	10	37	
Pyridoxine hydrochloride	3	58	42	
Pyridoxine hydrochloride	100	55	55	
Thiamin chloride	100	10	37	
Riboflavin	100	10	38	
Nicotinic acid	100	10	38	
Pantothenic acid	100	10	36	
Biotin	2	10	38	
<i>p</i> -Aminobenzoic acid	100	11	37	
Folic acid†	2	10	36	
<i>dl</i> -Alanine	1000	58	48	
<i>dl</i> -Alanine	5000	60	57	
<i>dl</i> - α -Aminoisobutyric acid	5000	10	37	
<i>dl</i> -Norvaline	5000	10	37	
<i>dl</i> -Valine	5000	9	37	
<i>dl</i> -Serine	5000	6	31	
<i>dl</i> -Threonine	5000	6	31	

* As in table 1.

† Potency 10,000.²³

TABLE 4
GROWTH INHIBITION BY COMPOUNDS RELATED IN STRUCTURE TO GLYCINE*

COMPOUND TESTED	INHIBITION	AMOUNT REQUIRED,† MG. PER 10 CC.	INHIBITION REVERSED BY	
			PYRIDOXINE HYDRO- CHLORIDE	<i>dl</i> -ALANINE
Glycine	+	2	+	+
β -Alanine	+	30	+	+
<i>dl</i> -Serine	+	15	+	+
<i>dl</i> -Threonine	+	15	+	+
<i>dl</i> -Alanine	—	(100)
Lactic acid	—	(30)
Creatine	—	(30)
Choline chloride	—	(30)
Benzoyl glycine	—	(30)
Glycollic acid	—	(30)

* All compounds were tested for their inhibitory properties by adding increasing amounts to the basal medium to which 0.5 γ of pyridoxine hydrochloride per 10 cc. was added. This amount of pyridoxine is sufficient to produce about one-half maximum growth of *S. lactis* R. under the test conditions.

† The amount of compound necessary to reduce growth to the level in the control tube (without pyridoxine) is given. Where the compound was not inhibitory, the highest level tested is given in parentheses.

pyridoxine does not permit growth in the absence of alanine. Glycine, threonine and serine (table 4) become inhibitory only when added in amounts considerably higher than those which are present in the hydrolyzed casein of the base medium.

TABLE 5
AMINO ACID REQUIREMENT OF *Streptococcus lactis* R.

AMINO ACID OMITTED	GALVANOMETER READING*	AMINO ACID OMITTED	GALVANOMETER READING*
None	51	<i>d</i> -Glutamic acid	0
Glycine	18	<i>dl</i> -Serine	0
<i>dl</i> -Alanine	0	<i>dl</i> -Threonine	0
<i>dl</i> -Alanine†	0	<i>l</i> -Arginine	0
<i>dl</i> -Valine	48	<i>l</i> -Histidine	49
<i>dl</i> -Leucine	5	<i>l</i> -Lysine	2
<i>dl</i> -Isoleucine	50	<i>l</i> -Proline	49
<i>l</i> -Phenylalanine	51	<i>l</i> -Hydroxyproline	51
<i>l</i> -Tyrosine	42	<i>dl</i> -Methionine	49
<i>l</i> -Aspartic acid	10	<i>l</i> -Tryptophane	0
		<i>l</i> -Cystine	20

* As in Table 1.

† 3.0 mg. pyridoxine hydrochloride (neutralized) added.

The extent to which these results apply to other lactic acid bacteria has not been extensively investigated. *S. lactis* 125 responds in exactly the same manner to additions of pyridoxine, alanine and glycine as does *S. lactis* R. Pyridoxine is also required for growth of *Lactobacillus casei*^{2, 3}; for this organism alanine does not replace pyridoxine. *L. casei* is likewise very resistant to glycine inhibition.

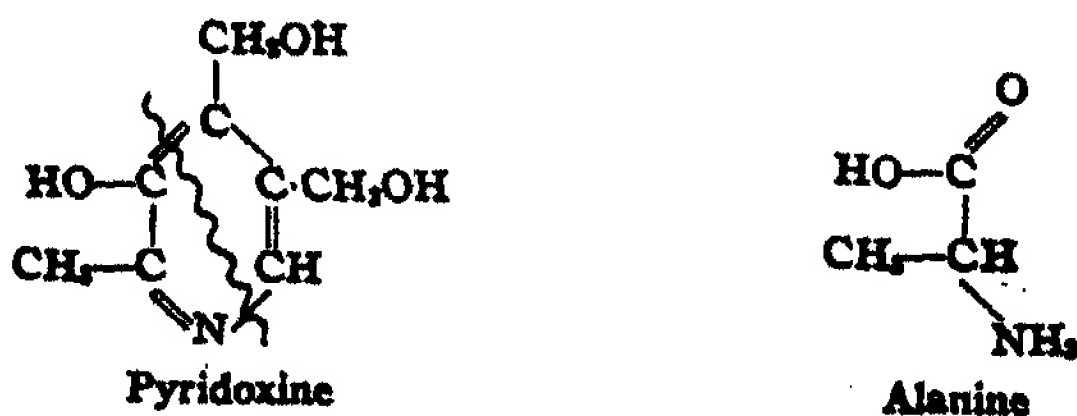
Discussion.—The inhibition of growth of *S. lactis* by glycine, serine, threonine or β -alanine and its removal by alanine is most easily explained as an example of metabolic interference of structurally similar compounds in the sense postulated by Woods⁸ and Fildes.⁹ Alanine is an essential amino acid, the presence of which is required for growth of this organism; the presence of large amounts of structurally similar amino acids blocks utilization of alanine. Addition of increased amounts of alanine while maintaining the concentration of the inhibiting substance constant again makes alanine available, by simple mass action. The fact that glycine, serine and threonine are themselves likewise essential for growth need not alter this conception; if they interfere with alanine utilization, growth is nevertheless prevented. McIlwain¹⁰ has shown that such interference may exist between amino acids and their sulfonic acid analogs, while existence of such interference between the sulfonamides and *p*-aminobenzoic acid⁸ or between pantothenic acid and pantoyl-aurine^{11, 12} is on firm experimental grounds. Similar cases of interference between amino acids have

been previously noted. Thus Gladstone,¹⁸ investigating the amino acid nutrition of *Bacillus anthracis*, found that valine or leucine added singly to a mixture of amino acids able to support growth without them completely prevented growth. The "toxic" effect of valine could be counteracted by addition of leucine, and vice versa. Similar interrelationships were found between valine and threonine, valine and α -aminobutyric acid, and threonine and serine.

The action of pyridoxine in alleviating toxicity due to glycine is not easily explained on this basis. Apparently, glycine blocks utilization of pyridoxine in some manner; additional amounts of pyridoxine counteract this effect. Indeed, counteraction of glycine inhibition by alanine and pyridoxine may be due to the same mechanism, since these compounds replace one another in their growth effect, presumably by permitting formation of the same physiologically active compound by the test organism. Somewhat analogous cases are known: methionine¹⁴ and the purine bases^{15, 16} can under certain circumstances reverse growth inhibitions produced by the sulfonamides, a result produced most effectively by *p*-aminobenzoic acid. In the absence of the sulfonamides, each of the above compounds may promote growth in the same manner.¹⁶

Various cases in which glycine^{17, 18, 19} and serine²⁰ were toxic when administered to animals have been reported. It would be of considerable interest to see if the toxicity of these compounds could be alleviated in these cases by additional amounts of pyridoxine or of alanine.

The complete replacement of pyridoxine for *S. lactis* by alanine deserves special comment. All experience to date has indicated that the B vitamins occur universally in all forms of living matter.^{21, 22} When an organism grows without added supplies, it does so because it is able to synthesize those supplies. According to this view, presence of sufficient quantities of alanine permits the synthesis by *S. lactis* of pyridoxine, or of the active substance derived from pyridoxine.^{4,6} In the absence of such concentrations of alanine, pyridoxine itself or its metabolites are necessary for growth. The mechanism by which alanine replaces pyridoxine is unknown, but it seems quite probable that it may serve as a direct precursor for pyridoxine in the following manner:



Organisms which are able to utilize alanine in place of pyridoxine would be

able to synthesize the remaining portion of the molecule and couple it with alanine.

Summary.—Alanine in sufficient concentration completely replaces pyridoxine for *Streptococcus lactis*. No other amino acid tested does this. Glycine inhibits growth; threonine, serine and β -alanine are inhibitory at higher levels. Inhibition by each of these substances is counteracted by addition of more pyridoxine to the medium. No other vitamin has this action. Such inhibition is also counteracted by alanine, but by no other amino acid. Possible explanations for these facts are discussed. Alanine may serve as a direct precursor of pyridoxine for *Streptococcus lactis*.

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STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES*

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It has been definitely established⁵ that the capacity of various microorganisms to produce antibiotic substances is widely distributed in nature. These substances are largely bacteriostatic and to a less extent bactericidal; some are also fungistatic and fungicidal. The antagonistic properties of an organism are not characteristic of a particular group or even species, but only of certain strains of given species. This was found to hold true, for example, for *Pseudomonas aeruginosa*, the first important antagonistic organism to receive attention. Some strains of this organism are able to produce the antibacterial agent pyocyanase, others form both pyocyanase and pyocyanin, whereas still others either are inactive or have only limited activity. This is also true of the spore-forming bacteria that possess antagonistic properties, only some of the strains being capable of inhibiting the growth of different bacteria. Much⁸ even suggested separation of the antagonistic strains of *Bacillus mycoides* into a special group, to be recognized as lysogenic strains. The antibacterial strains of spore-forming bacteria were shown⁴ to be characterized by specific physiological properties, such as production of H₂S on peptone media, failure to hydrolyze starch, and gram-negative nature of the cultures when 18 to 24 hours old; the inactive strains did not form H₂S, were able to hydrolyze starch, and were gram-positive in the 24 to 48-hour broth cultures.

The phenomenon of specificity is characteristic not only of bacteria but also of fungi. This is of particular interest in connection with the important organism *Penicillium notatum*, which produces the antibacterial agent penicillin. Some of the strains of this fungus produce penicillin abundantly, whereas others have only limited activity. The strain specificity of this organism applies not only to the quantitative production of penicillin, but also to qualitative differences. Some of the strains produce largely this important substance and very little of a second factor, which is also active against certain gram negative bacteria, such as *Escherichia coli*, and which has, therefore, been designated as the *E. coli* factor,⁶ penatin² or notatin,¹ whereas other strains show a reverse ratio in the production of these two active substances, especially under certain conditions of culture.⁶

In a survey of the distribution of antagonistic microorganisms in nature, 164 strains of different fungi were isolated from soils, composts and dust.⁶

Two species, *Aspergillus fumigatus* and *A. clavatus*, were selected for further study; the first was represented by 16 isolations and the second by 15 strains. Although morphologically the same, the various strains differed greatly in their capacity to produce the specific antibacterial substances fumigacin and clavacin. The differences appeared to be quantitative rather than qualitative in nature; although the amount of the active substance produced on the same medium and under the same conditions varied, the nature of the substance was apparently the same for all strains of the particular organism.

TABLE 1
GROWTH AND PRODUCTION OF CLAVACIN BY *A. clavatus* 129
Shallow Layers (250-Ml. Portions) in 1-Liter Flasks, at 28°C.

AGE OF CULTURE, DAYS	REACTION OF MEDIUM, pH	GLUCOSE CONSUMED, G. PER LITER	NO ₃ -N IN MEDIUM, MG. PER LITER		ACTIVITY OF CULTURE, <i>E. coli</i> UNITS	CLAVACIN ISOLATED	
			LEFT	CONSUMED		YIELD, G. PER LITER	ACTIVITY OF MATERIAL, ^a <i>E. coli</i> UNITS
0	4.6	..	51.2	0
3	4.9	1.50	28.4	22.8	30	0.27	120,000
5	5.1	2.82	7.5	43.7	60	0.52	100,000
7	5.0	3.40	4.4	46.8	100	0.63	200,000
10	5.1	3.86 ^b	3.0	48.2	100	0.71	200,000
14	5.5	3.86	2.9	48.3	75	0.72	10,000
21	5.9	3.86	30	0.66	600
26	6.6	3.86	10
36	6.5	3.86	0	0.62	200

^a A unit of activity is the ratio of 10 to the amount of material required for 10 ml. of nutrient agar, in order to inhibit completely the growth of the test organisms.

^b Complete consumption of sugar.

In order to throw further light upon the influence of strain specificity in connection with the production of antibiotic substances, the results of the following experiments with *A. clavatus* are reported here. Of the 15 strains employed in these studies, 3 (Nos. 129, 130, 164) were isolated in this laboratory from composts and 12 were received from Dr. Charles Thom, of Washington, who obtained them from different sources. A simple synthetic medium, usually designated as Czapek-Dox,⁶ was used. The course of growth of one of the active strains of this organism, as measured by sugar consumption and nitrogen assimilation, and the rate of production of the antibiotic substance were brought out in table 1. The highest antibacterial activity of the culture corresponded with the maximum growth period, as indicated by the complete utilization of the sugar and maximum consumption of the nitrogen. One may, therefore, conclude that the formation of the antibacterial substance is not a result of autolysis of the fungus mycelium, since after the maximum growth of the fungus was attained, the activity was rapidly reduced. Whether the substance is

produced during the breakdown of the sugar or is synthesized in the cell material and excreted into the medium still remains to be determined.

The active substance, clavacin, was isolated from a definite volume of culture medium at the various periods of growth, by the use of a method described elsewhere.⁷ The yield of the material thus isolated reached a

TABLE 2
PRODUCTION OF CLAVACIN BY 15 STRAINS OF *A. clavatus*

NO. OF STRAIN	IN-CUBATION, DAYS	pH	ACTIVITY OF CULTURE		YIELD, G. PER LITER	ISOLATED CLAVACIN ^a	
			<i>E. coli</i> UNITS	<i>B. subtilis</i> UNITS		ACTIVITY, UNITS <i>E. coli</i>	PER 1 G. <i>B. subtilis</i>
120	5	6.5	0	0	0.016	25,000	75,000
120	14	8.4	0	0
121	5	4.2	75	75	1.442	1,000,000	1,200,000
121	13	6.7	100	300
122	5	4.5	0	0	0.035	< 4,000	< 4,000
122	13	8.0	0	0
123	5	4.6	20	20	0.467	120,000	120,000
123	13	4.5	75	100
124	5	6.2	0	0	0.016	< 8,000	< 8,000
124	13	8.4	0	0
125	5	3.2	0	0	0.248	600	10,000
125	13	3.9	0	0
126	5	6.3	0	0	0.039	< 20,000	< 20,000
126	13	8.2	0	0
127	5	7.4	0	0	0.007	< 8,000	< 8,000
127	13	8.1	0	0
128	5	6.7	0	0	0.017	7,000	14,000
128	13	8.0	0	0
129	5	3.6	100	100	0.950	400,000	3,000,000
129	7	4.4	150	250
129	15	6.8	75	100
129T	5	6.6	20	10	0.512	80,000	120,000
129T	15	5.9	75	75
130	5	4.8	< 10	< 10	0.323	500,000	1,400,000
130	13	4.7	20	30
130T	5	6.9	< 10	30	0.050	< 4,000	40,000
130T	15	7.9	< 10	< 10
131	5	6.9	< 10	30	0.035	5,000	25,000
131	15	7.8	< 10	< 10
164	5	4.3	30	25	0.430	1,000,000	2,000,000
164	7	4.6	90	100

^a Eight-day-old culture used for the extraction of the clavacin.

maximum in 7-10 days, corresponding to the maximum activity of the culture itself. After the maximum was reached the yield of extracted material remained constant, but its activity diminished rapidly, parallel to the reduction in the activity of the culture medium of the organism. This emphasizes the fact that the clavacin produced by the fungus undergoes in the culture some chemical change which results in the destruction of

its activity. The crude, isolated clavacin is also rather unstable, especially under certain conditions as at an alkaline reaction or on complete drying. The nature of the chemical changes thus produced is a matter for further study.

The production of clavacin by the 15 different strains of *Aspergillus clavatus* is illustrated in table 2. These strains differed greatly in their metabolism, as measured, for example, by changes in the pH values of the medium: the reaction became alkaline in some cases, but in others it remained distinctly acid, in the same medium and under the same conditions of culture. There were other marked differences in the growth of the various strains, which need only be mentioned here, such as the intensity of the odor produced, the pigmentation (yellow to orange) of the cultures, and the abundance and rate of appearance of mycelium and of spores. The most significant difference, however, was in the antibacterial activity of the strains, some of which were highly active, whereas others had no activity at all. The three strains that were isolated from stable manure, namely, Nos. 129, 130, 164, produced a highly active substance; the actual yield of this substance differed, however, and the difference was sufficient to account for the variation in the activity of the culture medium. Only three of the cultures obtained from Dr. Thom, namely, Nos. 121, 123 and 129T, gave a high activity and a fair yield of antibacterial substances. The clavacin isolated for the different active strains appeared to be the same, as shown by chemical properties and by its antibacterial spectrum or the specific action against different bacteria. There was only one exception, that of strain No. 130T; whereas all the other strains produced a substance which was 2-5 times as active against *Bacillus subtilis* as against *Escherichia coli*, this strain had little, if any, activity against *E. coli* as compared with a fair activity against *B. subtilis*. On further study, this strain was found to comprise two forms, which may explain the difference in the apparent activity of the antibacterial substance produced.

It is interesting to note that, whereas the reaction of the medium of all the six active strains remained distinctly acid, the reaction of the inactive strains, with the exception of No. 125, changed to alkaline. When large volumes of the culture medium of all the strains were treated alike for the isolation of the clavacin, it was found that even some of the inactive strains yielded some active substance. A liter of medium was treated with Norit A, which was then extracted with ether and chloroform. Some of the inactive strains gave some clavacin, possessing fair activity, as, for example, No. 120; other strains, however, gave a good yield of material with only limited antibacterial activity, as No. 125. Whether this fact proves that even the inactive strains may produce clavacin, which becomes inactivated as a result of unfavorable changes in the medium (reaction, for example), still remains to be determined.

These results warrant the conclusion that a single species of a fungus contains many strains that vary greatly in their capacity to produce antibiotic or antibacterial substances. The variation is both quantitative and qualitative, though the active substance produced by the different strains is apparently the same, chemically and biologically. This tends to emphasize the danger of drawing conclusions regarding the antibacterial activity of an organism based upon type cultures obtained from

TABLE 3
BACTERIOSTATIC ACTIVITY OF THREE CLAVACIN PREPARATIONS

On Basis of 1 G. of Dry Material			
TEST ORGANISM	NO. 121	NO. 123	NO. 129
<i>Bacillus subtilis</i>	300,000	250,000	275,000
<i>Bacillus megatherium</i>	150,000	125,000	140,000
<i>Bacillus mycoides</i>	100,000	85,000	100,000
<i>Bacillus cereus</i>	150,000	125,000	100,000
<i>Sarcina lutea</i>	300,000	250,000	275,000
<i>Staphylococcus aureus</i> W2	150,000	125,000	100,000
<i>Staphylococcus aureus</i> D	200,000	165,000	170,000
<i>Staphylococcus aureus</i> H	150,000	83,000	100,000
<i>Staphylococcus aureus</i> W1	150,000	125,000	130,000
<i>Salmonella schottmuelleri</i>	300,000	250,000	200,000
<i>Vibrio cholera-suis</i>	350,000	300,000	270,000
<i>Salmonella</i> sp., Breslau strain	250,000	165,000	265,000
<i>Aerobacter aerogenes</i>	75,000	63,000	80,000
<i>Escherichia coli</i>	175,000	165,000	230,000
<i>Shigella dysenteriae</i> ^a	250,000
<i>Shigella paradysenteriae</i> ^a	210,000

^a Mixed lot of clavacin.

collections. Wilkins and Harris⁸ recently reported, for example, on the basis of tests with one culture that *A. clavatus* does not produce any bacteriostatic substance, whereas 40 per cent of all the *Aspergilli* tested produced such substances. Had other strains of these organisms been employed, totally different results undoubtedly would have been obtained.

It has been shown previously⁷ that clavacin is almost as active against gram-negative bacteria as against gram-positive organisms. In order to establish whether this is true for preparations isolated from the different active strains of *A. clavatus*, the results of a typical experiment are presented in table 3. Three clavacin preparations were obtained and found to be alike in their action against a number of gram-positive and gram-negative bacteria, with only minor variations. In all cases, *Aerobacter aerogenes* was less sensitive to the action of clavacin than was *E. coli*, and *B. mycoides* and *B. cereus* were less sensitive than was *B. subtilis*.

Summary.—A study was made of the production of the antibacterial substance clavacin by 15 different strains of the fungus *Aspergillus clavatus*.

The formation of clavacin in the culture medium corresponded with the maximum growth, as measured by the complete consumption of the sugar in the medium and by maximum nitrogen utilization.

After the maximum antibacterial activity had been reached in the medium, the activity of the clavacin was rapidly destroyed. This was found to be due not to the disappearance of the substance itself from the medium but to its inactivation.

Only six of the fifteen strains of *A. clavatus* produced considerable amounts of clavacin. The remaining nine strains gave only traces of the active substance.

The clavacin produced by the different active strains of *A. clavatus* was found to be the same biologically, as shown by its activity against different bacteria, and apparently the same chemically, as shown by solubility studies and chemical behavior.

Attention is directed to too hasty generalizations concerning the ability of certain fungus species to produce antibacterial substances, based upon the study of single strains of a given organism.

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THE DETERMINATION OF L.D.50 AND ITS SAMPLING ERROR IN BIO-ASSAY

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In the bio-assay problem we have two methods in the literature: (1) the probit method which has been actively promoted by C. I. Bliss, though suggested in its essentials by Gaddum, and (2) the 50% end-point method of Reed and Muench, which, again, was developed from earlier sugges-

tions.¹ Both of these methods depend fundamentally on the assumption that the probability of an effect being realized in an infinite population to each of which a dose D has been administered is given by the probability integral

$$P = \frac{1}{\sqrt{2\pi}\sigma} \int_{-\infty}^x e^{-\frac{(x-\mu)^2}{2\sigma^2}} dx, \quad x = \log D, \quad (1)$$

where $\mu = \text{L.D.50}$ is the dose measured as the natural logarithm of D which affects just one-half of the population, and σ is a measure of the scatter of the effect, a sort of inverse of the homogeneity of the population with respect to the effect; and both methods depend further on an arithmetic-graphic treatment to smooth out statistical fluctuations, due to small numbers in the experimental samples.

To examine a number of matters to which we desire to call attention it will be simpler to replace the probability-integral (1) by the growth curve which between wide limits does not depart seriously therefrom; indeed it is known that the value of P does not precisely follow (1), sometimes departing considerably from it, and it is by no means certain that the growth curve would not fit the observations as well as the probability integral curve. If D be the dose we may then write

$$P = \frac{D^{2\alpha}}{D^{2\alpha} + e^{2\alpha\gamma}} = \frac{1}{1 + e^{2\alpha(\gamma - x)}} = \frac{1}{2} + \frac{1}{2} \tanh \alpha (x - \gamma)$$

where $x = \log D$ and \tanh is the hyperbolic tangent. The constant $\gamma = \text{L.D.50}$; the constant α is a measure of the homogeneity of the reaction of the population. If P_1 and P_2 be the percentages of the population which respond to doses D_1 and $D_2 = aD_1$ so that $x_2 = x_1 + \log a = x_1 + c$ we have

$$\frac{1}{P_1} = 1 + e^{2\alpha(\gamma - x_1)}, \quad \frac{1}{P_2} = 1 + e^{2\alpha(\gamma - x_1 - c)}$$

$$\log \frac{Q_1}{P_1} = 2\alpha(\gamma - x_1), \quad \log \frac{Q_2}{P_2} = 2\alpha(\gamma - x_1 - c)$$

where $Q_i = 1 - P_i$ and

$$\alpha = \frac{1}{2c} \left[\log \frac{Q_1}{P_1} - \log \frac{Q_2}{P_2} \right], \quad \gamma = \frac{x_1 + x_2}{2} + \frac{1}{4\alpha} \left[\log \frac{Q_1}{P_1} + \log \frac{Q_2}{P_2} \right]. \quad (2)$$

If then we take $P_1 = 0.25$ and $P_2 = 0.75$ we find

$$\alpha = \frac{1}{c} \log 3 = \frac{1.099}{c} \quad \text{or} \quad c = \frac{1.099}{\alpha}$$

so that c which is the difference in the natural logarithms of the two doses

which affect 75 and 25% of the population, respectively, is inversely proportional to α .

In the experimental case x_1 and x_2 are known and P_1 and P_2 are observed; from them α and γ may be computed. In many cases α is not desired, but only $\gamma = \text{L.D.50}$; we may then write

$$\gamma = \frac{x_1 + x_2}{2} + \frac{c \log (Q_1/P_1) + \log (Q_2/P_2)}{2 \log (Q_1/P_1) - \log (Q_2/P_2)}, \quad c = x_2 - x_1. \quad (3)$$

The standard deviation of γ may be obtained by the usual method of differentiation.

$$\delta\gamma = \frac{c}{2} \delta \frac{\log (Q_1/P_1) + \log (Q_2/P_2)}{\log (Q_1/P_1) - \log (Q_2/P_2)} = c \frac{\frac{\delta P_1}{P_1 Q_1} \log \frac{Q_2}{P_2} - \frac{\delta P_2}{P_2 Q_2} \log \frac{Q_1}{P_1}}{[\log (Q_1/P_1) - \log (Q_2/P_2)]^2}$$

As P_1 and P_2 are subject to sampling variations of PQ/n , when n is the number of animals in each of the samples (ordinarily the same) to which the two doses are given, and as those sampling variations are independent (non-correlated), we may square, neglecting the product $\delta P_1 \delta P_2$ and substitute for $(\delta P)^2$ the value PQ/n . Then²

$$\sigma_\gamma^2 = \frac{c^2}{n} \frac{\frac{1}{P_1 Q_1} \left(\log \frac{Q_2}{P_2} \right)^2 + \frac{1}{P_2 Q_2} \left(\log \frac{Q_1}{P_1} \right)^2}{[\log (Q_1/P_1) - \log (Q_2/P_2)]^4}. \quad (4)$$

Two biologicals which are standardized at the L.D.50 points will not stay standardized for other dosages unless the two values of α are the same. Thus if $\alpha_1 > \alpha_2$, the first will be relatively stronger than the second above the L.D.50 point, and relatively weaker below it. The standard deviation of α is therefore for the purpose of determining whether the values of α are significantly different. By the process of differentiation,

$$\sigma_\alpha^2 = \frac{1}{4nc^2} \left[\frac{1}{P_1 Q_1} + \frac{1}{P_2 Q_2} \right]. \quad (5)$$

If there be two values of α each determined by two doses upon n animals

$$\sigma_{(\alpha - \alpha')}^2 = \frac{1}{4nc^2} \left[\frac{1}{P_1 Q_1} + \frac{1}{P_2 Q_2} + \frac{1}{P'_1 Q'_1} + \frac{1}{P'_2 Q'_2} \right].$$

The standard deviation of the difference $\gamma - \gamma'$ is

$$\sigma_{(\gamma - \gamma')}^2 = \frac{1}{4n} \left\{ \frac{1}{\alpha^2} \left[\frac{1}{P_1 Q_1} \left(\log \frac{Q_2}{P_2} \right)^2 + \frac{1}{P_2 Q_2} \left(\log \frac{Q_1}{P_1} \right)^2 \right] + \frac{1}{\alpha'^2} \left[\frac{1}{P'_1 Q'_1} \left(\log \frac{Q'_2}{P'_2} \right)^2 + \frac{1}{P'_2 Q'_2} \left(\log \frac{Q'_1}{P'_1} \right)^2 \right] \right\}.$$

If the values of α and α' are different, the result is of less value than if they are the same, because standardization at the L.D.50 points does not ensure standardization at other points.³

In this analysis the standard errors of sampling have been obtained as usual by the process of differentiation as is the case with such formulas as $\sigma \log x = \sigma_x/x$. It may, however, be observed that if x may be 0 with a finite probability, $\log x$ must have an infinite value with a finite probability and its standard deviation, strictly considered, must be infinite. Thus such approximations are justifiable only if the probabilities of infinite values are so small that we are willing to disregard them altogether. It is very difficult to discuss this matter in general but an illustration is extremely germane to an understanding of what happens when the numbers n are not very large. Suppose we have two samples of $n = 10$. Then the values of γ and of α and of their standard deviations may be computed for any values of $s_1 = 10P_1$ and $s_2 = 10P_2$ that may be observed on giving the two doses to the two samples. It will be observed from table 1, as is clear from formulas (2)–(5), that many entries in the table are valueless so far as concerns bio-assay. For example, if $s_1 = 0$, $P_1 = 0$ and s_2 is anything other than 10 or 0, formula (3) gives " γ " = 1.0, which means that $\gamma = \text{L.D.50} = x_2$, although except when $s_2 = 5$ there is no evidence that γ should be equal to x_2 and the standard error $\sigma_{\gamma} = \infty$ in these cases only emphasizes this fact. Again, the values of " γ " and " α " when in the diagonal for which $s_1 = s_2$ are useless. Moreover, we are generally dealing with a biological for which we know on *a priori* grounds that negative values of " α " are without meaning. Nevertheless all these embarrassing cases may arise with finite probability from sampling fluctuations no matter what the true values of P_1 and P_2 in the infinite population may be.⁴

In any particular problem of determination of L.D. 50 we should probably know that for the biologicals used $P_2 > P_1$ in the universe and would not be interested in the useless values computed for $s_1 = 0$, $s_2 = 10$ or $s_1 \geq s_2$. While there would be definite values of " γ " and of " α " obtainable from this universe if P_1 and P_2 were known, we should actually obtain in any one run some one of the values entered in the table, and in the general case it is not necessary that the true universe values appear in the table. Moreover, as the formulas (2) and (3) when $P_1 = s_1/n$ and $P_2 = s_2/n$ are not linear in s_1 and s_2 it cannot be expected that the average of the values obtained when the experiment is run a large number of times are the values in the universe.⁵ In like manner the values of σ_{γ}^2 and σ_{α}^2 which we should have to use would be those entered in the table corresponding to the acceptable observed values of s_1 and s_2 and would be very variable, and moreover if the experiment were run many times the scatter of " γ " and " α " computed from the different values of " γ " and " α " that were

TABLE 1

VALUES OF "γ" = $\left[\gamma - \frac{x_1 + x_2}{2} \right]_c^2$ AND "α" = 2cα, THE REMAINDER TO BE FILLED IN BY SYMMETRY									
	$P_1 = 1.0$ $s_1 = 10$	$P_2 = 0.9$ $s_2 = 9$	$P_3 = 0.8$ $s_3 = 8$	$P_4 = 0.7$ $s_4 = 7$	$P_5 = 0.6$ $s_5 = 6$	$P_6 = 0.5$ $s_6 = 5$	$P_7 = 0.4$ $s_7 = 4$		
$P_1 = 0.0$?? ± ??	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00		
$s_1 = 0$	∞ ± ∞	∞ ± ∞	∞ ± ∞	∞ ± ∞	∞ ± ∞	∞ ± ∞	∞ ± ∞		
$P_1 = 0.1$	-1.00 ± 0.00	0 ± 0.34	0.23 ± 0.35	0.44 ± 0.38	0.69 ± 0.44	1.00 ± 0.58	1.45 ± 0.92		
$s_1 = 1$	∞ ± ∞	4.39 ± 1.49	3.58 ± 1.32	3.04 ± 1.26	2.60 ± 1.24	2.20 ± 1.23	1.79 ± 1.24		
$P_1 = 0.2$	-1.00 ± 0.00	-0.23 ± 0.35	0 ± 0.40	0.24 ± 0.47	0.55 ± 0.59	1.00 ± 0.91	1.83 ± 1.98		
$s_1 = 2$	∞ ± ∞	3.58 ± 1.32	2.77 ± 1.12	2.23 ± 1.05	1.79 ± 1.02	1.39 ± 1.01	0.98 ± 1.02		
$P_1 = 0.3$	-1.00 ± 0.00	-0.44 ± 0.38	-0.24 ± 0.47	0 ± 0.58	0.35 ± 0.78	1.00 ± 1.49	2.84 ± 6.29		
$s_1 = 3$	∞ ± ∞	3.04 ± 1.26	2.23 ± 1.05	1.70 ± 0.98	1.25 ± 0.94	0.85 ± 0.93	0.44 ± 0.94		
$P_1 = 0.4$	-1.00 ± 0.00	-0.69 ± 0.44	-0.55 ± 0.59	-0.35 ± 0.78	0 ± 1.12	1.00 ± 3.12	∞ ± ∞		
$s_1 = 4$	∞ ± ∞	2.60 ± 1.24	1.79 ± 1.02	1.25 ± 0.94	0.81 ± 0.91	0.40 ± 0.90	0 ± 0.91		
$P_1 = 0.5$	-1.00 ± 0.00	-1.00 ± 0.58	-1.00 ± 0.91	-1.00 ± 1.49	-1.00 ± 3.12	?? ± ??	-1.00 ± 3.12		
$s_1 = 5$	∞ ± ∞	2.20 ± 1.23	1.39 ± 1.01	0.85 ± 0.93	0.41 ± 0.90	0 ± 0.89	-0.41 ± 0.90		
$P_1 = 0.6$	-1.00 ± 0.00	-1.45 ± 0.92	-1.83 ± 1.98	-2.84 ± 6.29	-	1.00 ± 3.12	0 ± 1.12		
$s_1 = 6$	∞ ± ∞	1.79 ± 1.24	0.98 ± 1.02	0.44 ± 0.94	0 ± 0.91	-0.41 ± 0.90	-0.81 ± 0.91		
$P_1 = 0.7$	-1.00 ± 0.00	-2.26 ± 1.93	-4.14 ± 8.04	-	2.84 ± 6.29	1.00 ± 1.49	0.35 ± 0.78		
$s_1 = 7$	∞ ± ∞	1.35 ± 1.26	0.54 ± 1.05	0 ± 0.98	-0.44 ± 0.95	-0.85 ± 0.94	-1.25 ± 0.94		
$P_1 = 0.8$	-1.00 ± 0.00	-4.42 ± 6.90	-	4.14 ± 8.04	1.83 ± 1.98	1.00 ± 0.91	0.55 ± 0.59		
$s_1 = 8$	∞ ± ∞	0.81 ± 1.32	0 ± 1.12	-0.54 ± 1.05	-0.98 ± 1.02	-1.39 ± 1.01	-1.79 ± 1.02		
$P_1 = 0.9$	-1.00 ± 0.00	-	4.42 ± 6.90	2.26 ± 1.93	1.45 ± 0.93	1.00 ± 0.58	0.69 ± 0.44		
$s_1 = 9$	∞ ± ∞	0 ± 1.49	-0.81 ± 1.32	-1.35 ± 1.26	-1.79 ± 1.24	-2.20 ± 1.23	-2.60 ± 1.24		
$P_1 = 1.0$?? ± ??	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00		
$s_1 = 10$?? ± ??	-	∞ ± ∞	-	∞ ± ∞	-	∞ ± ∞		

figured from the observations would not in general be the same as, and might be very different from, the values proper to the universe.⁶

From such considerations it is clear that values of n considerably larger than 10 are necessary to establish that degree of stability in the results figured from the observations which is desirable in any bio-assay. For all values of n the values of " γ " and " α " are as given in the table when the margins are read for P_1 and P_2 (rather than for s_1 and s_2) but the standard deviations would have to be reduced by multiplying by $\sqrt{10/n}$. Furthermore, when n is not 10 the observed values of P may differ from the values entered in the margins and it would be necessary to interpolate by double entry⁷ in the table in order to get the values of " γ " and " α ." The values of " γ " and " α " and of their standard errors are independent of the logarithmic base, whether e or 10, and the values of γ and α may be obtained in either type by the formulas

$$\gamma = \frac{x_1 + x_2}{2} + \frac{c}{2} " \gamma ", \quad \alpha = \frac{" \alpha "}{2c}$$

$$\sigma_\gamma = \frac{c}{2} \sigma_{" \gamma " } \sqrt{10/n}, \quad \sigma_\alpha = \frac{\sigma_{" \alpha "}}{2c}$$

where $x_1, x_2, c = x_2 - x_1$ are expressed in logarithms to any base. If one wishes to return finally to the actual dosage, D , one has merely to write $L.D.50 = e^\gamma$ or $L.D.50 = 10^\gamma$ according to the base used.

⁶ Gaddum, J. H., *Med. Res. Council, Spec. Rep. Series*, No. 183 (1933). Bliss, C. I., *Ann. Applied Biol.*, 22, 134-167 (1935). Reed, L. J., and Muench, H., *Amer. Jour. Hygiene*, 27, 493-497 (1938). There is a review of the subject by J. O. Irwin, *Suppl. Jour. Roy. Statistical Soc. (London)*, 4, 1-60 (1937).

⁷ Thus, in particular, if we have $P_1 = 0.30$ for a given dose D_1 and $P_2 = 0.75$ for a dose D_2 twice as large, the value of c is $\log 2 = 0.693$, $P_1 Q_1 = 0.21$, $P_2 Q_2 = 0.1875$, $Q_1/P_1 = 1/2$, $Q_2/P_2 = 1/2$, $\log (1/2) = 0.847$, $\log (1/3) = -1.099$ and

$$\sigma_\gamma^2 = \frac{(0.693)^2}{n} \frac{\frac{1}{0.21} (1.099)^2 + \frac{1}{0.1875} (0.847)^2}{[0.847 + 1.099]^2} = \frac{0.321}{n}$$

$$\gamma = \frac{x_1 + x_2}{2} - 0.0446 = \sqrt{\frac{0.321}{n}}.$$

The term $1/2(x_1 + x_2)$ is the mean logarithmic dose and the 50% end-point is short of it by the amount 0.0446. If n were 20, the standard error would be 0.126; so that we should expect in two-thirds of the runs the value of L.D.50 would lie between 0.081 above and 0.171 below the mean logarithmic dose.

⁸ Suppose we have dilutions 1 with $P_2 = 0.75$ and $1/2$ with $P_1 = 0.30$ of one biological and dilutions $1/2$ with $P_2' = 0.75$ and $1/4$ with $P_1' = 0.40$ of a second. We take $x_1 = 0$, $x_2 = 0.693$, $x_2' = 0$, $x_1' = -0.693$. The value of γ is $1/2(0.693) - 0.0446$ as above with $\sigma_\gamma^2 = 0.321/n$. The value of γ' is $-1/2(0.693) - 0.159$. The difference between the values is $0.693 - 0.045 + 0.159 = 0.807$. Thus at the L.D.50 points the ratio of

strength is the antilogarithm of 0.807 base e , or 2.24. The values of α are $\alpha = 1.40$ and $\alpha' = 1.09$. The standard errors, if $n = 20$, are $\sigma_{\alpha}^2 = 0.263$, $\sigma_{\alpha'}^2 = 0.247$; that of the difference being $\sigma^2 = 0.510$ or $\sigma = 0.71$ which is considerably larger than the difference $\alpha - \alpha' = 0.31$ and indicates that, so far as the test goes, α and α' may be taken as the same. (If we were to take the "best" value for α on the assumption that they were really the same we should then weight the two values according to the reciprocal of the squares of their standard errors, but these are so nearly alike that we could well take the simple mean 1.24.) The value of $\sigma^2_{\gamma-\gamma'}$ for $n = 20$ is $0.321/n + 0.554/n = 0.875/n = 0.0438$; hence the difference may be written 0.807 ± 0.209 and is distinctly significant. The relative intensity 2.24 may be given a factor or quotient of the antilogarithm (base e) which is 1.23 or its reciprocal 0.81 or about 21% or 2.24 ± 0.47 .

⁴ If it be assumed that $P_1 = 0.20$ and $P_2 = 0.80$ in the universe the table of probabilities is (in part, the remainder to be filled in by symmetry):

	$s_2 = 10$	$s_2 = 9$	$s_2 = 8$	$s_2 = 7$	$s_2 = 6$	$s_2 = 5$	$s_2 = 4$
$s_1 = 0$	0.0115	0.0288	0.0324	0.0216	0.0095	0.0028	0.0006
$s_1 = 1$	0.0288	0.0721	0.0811	0.0540	0.0236	0.0071	0.0015
$s_1 = 2$	0.0324	0.0811	0.0912	0.0608	0.0266	0.0080	0.0017
$s_1 = 3$	0.0216	0.0540	0.0608	0.0405	0.0177	0.0053	0.0011
$s_1 = 4$	0.0095	0.0236	0.0266	0.0177	0.0078	0.0023	0.0005
$s_1 = 5$	0.0028	0.0071	0.0080	0.0053	0.0023	0.0007	0.0002
$s_1 = 6$	0.0006	0.0015	0.0017	0.0011	0.0005	0.0002	0.0000
$s_1 = 7$	0.0001	0.0002	0.0002	0.0002	0.0001	0.0000	0.0000
$s_1 = 8,$	$s_1 = 9,$	$s_1 = 10$	give for all s_2 's the probability 0.0000 to four figures.				

In this case the troublesome margins for $s_1 = 0$ and for $s_2 = 10$ have a total probability of 0.2033, the troublesome diagonal has an additional probability of 0.0020 and the total probability for $s_1 > s_2$ is 0.0005. This makes a grand total of 0.2058. Had we assumed the $P_1 = 0.40$ and $P_2 = 0.60$ we should have had a smaller probability (0.0120) in the margins for $s_1 = 0$ and $s_2 = 10$, but a larger value (0.2445) for the diagonal $s_1 = s_2$ and below, making an even larger probability of 0.2565 for the cells which are meaningless for the problem of assay.

⁵ To find the universe values of γ and α from a large number of runs one would have to add the different s_1 's, the different s_2 's and the different n 's to obtain the probabilities P_1 and P_2 to be used in the formula.

⁶ Thus, if, for the universe, $P_1 = 0.20$, $P_2 = 0.80$, the value of σ^2_{γ} would be 0.162 for $n = 10$ by formula (4), but the different values of " γ " from the experiments would have the probabilities given in the table,⁴ and the actual value of σ^2_{γ} observed in the long run would be 0.215 if we reject the experiments which have unusable constellations (s_1, s_2); and the value of σ^2_{γ} from the observed distribution of usable values would be 0.86 instead of the value 1.25 given by formula (5).

⁷ If $P_1' = P_1 + \theta_1 \Delta P_1$ and $P_2' = P_2 + \theta_2 \Delta P_2$, the interpolation formula is

$$f(P_1', P_2') = f(P_1, P_2) + \theta_1 [f(P_1 + \Delta P_1, P_2) - f(P_1, P_2)] + \theta_2 [f(P_1, P_2 + \Delta P_2) - f(P_1, P_2)].$$

For a table with such large differences as table 1, the interpolation could not be expected to give a very accurate determination of $f(P_1', P_2')$ but might be adequate.

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GONIOLITHON AND NEOGONIOLITHON: TWO GENERA OF CRUSTACEOUS CORALLINE ALGAE

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Foslie, in his earlier studies of the crustaceous Corallines, proposed a new genus *Goniolithon* (see Foslie, "Syst. Surv. Lithoth.," 5 (1898), with scant description. He also described two subgenera, *Eugoniolithon*, apparently from his designation, the typical subgenus, and *Cladolithon*, the latter subgenus to include the fruticulose species. The type and only species of *Eugoniolithon* was the *Lithothamnion papillosum* Zanardini (as described by Hauck). This certainly is to be considered as the type of the genus *Goniolithon* of 1898. The only species referred to the subgenus *Cladolithon*, which in a footnote Foslie describes as a doubtful subgenus, is the *Lithophyllum byssoides* (Lam'k) Foslie, at that time a heterogeneous mixture in the mind of Foslie, as will be indicated later. Taking *G. papillosum* (Zan.) Foslie as the type of *Goniolithon*, the genus is a perfectly distinct entity, being made up of strictly monostromatic layers (without cover cells or perithallus of any kind) and belongs therefore to the subfamily *Mastophoreae* of the *Corallinaceae Crustaceae*, all of whose members have monostromatic (at times symmetrically di-polystromatic?) sterile frond structure, thus differing from all other *Corallinaceae*. The sterile fronds also have a way of obliquely and lamellately proliferating, and these proliferations produce superposed and distinct layers in *Litholepis* and *Lithoporella*, but are agglutinated into a solid frond in the original *Goniolithon*.

Later, in 1898 ("List of Species of the Lithoth.," p. 8) Foslie changes the name of the subgenus I. *Eugoniolithon* to subgenus I. *Lepidomorphum*, listing 4 species of which *G. papillosum* is first mentioned, while under subgenus II. *Cladolithon* he mentions *G. byssoides* first of 18 species and *G. moluccense* second. In 1900, in his "Revised Systematical Survey of the Melobesieae" (p. 15), Foslie rejects his earlier *Goniolithon* (of 1898) and proposes an entirely new *Goniolithon* distinguished by having the tetrasporangia distributed over the floor of the conceptacle and (incidentally

because he makes no mention of their occurrence) with heterocysts scattered through the perithallus. This new *Goniolithon* may be considered to have been founded on the *Lithothamnium moluccense* Foslie, since Foslie stated that his *G. byssoides* of 1898 was intended to refer to his *G. moluccense*. At any rate, Foslie has proposed two distinct genera under *Goniolithon*, the original in 1898 with the type *G. papillosum* (Zan.) Foslie and the other partly visualized under the doubtful subgenus *Cladolithon* in 1898 but only distinctly segregated about 1904 ("Siboga Exp.," Mon. LXI, 45 (1904)) and finally properly delimited (by the exclusion of *Hydrolithon*) in 1909 ("Algol. Notis.," VI).

Two facts stand out clearly: (1) that Foslie completely rejected his *Goniolithon* of early 1898; and (2) that Foslie gradually evolved a new *Goniolithon*, finally brought to full expression in 1909. The two genera are complete and fairly independent establishments, and no single species of *Goniolithon* No. 1 survived into *Goniolithon* No. 2. This condition might have been stabilized by an international botanical congress without change of name, were it not for the fact that the type of *Goniolithon* No. 1 (*Goniolithon papillosum*) is a generic type of its own, distinct not only from *Goniolithon* No. 2 but from all other genera. The proposal which seems most logical is to recognize *Goniolithon* No. 1, with type species *G. papillosum*, and to rename *Goniolithon* No. 2, *Neogoniolithon* nov. nom., with such a species as is most characteristic and well described, as *G. Fosliei* (Heydr.) Foslie, for the type. The consequent changes are enumerated in the following paragraphs.

***Goniolithon* Foslie (restr. to subfamily *Mastophoreae*)**

Fronds crustaceous to decidedly thick, composed of separate monostromatic layers arising from those immediately below by obliquely lamelliform proliferation and becoming cemented together; conceptacles simple (without thickening of frond tissue) in all types, opening by a simple pore.

Foslie, "Systematical Survey of the Lithothamnia," 5 (1898), as to subgenus I. *Eugoniolithon* (non aliorum, p.p. exceptorum). (!) means that the type specimen has been examined by, at least, one of us (Setchell).

***Goniolithon papillosum* (Zan.) Foslie (type!)**

Lithophyllum papillosum Zanardini, "Saggio," 43 (1843) (!) (*sine descr.*) Hauck, "Meeresalgen, 272, pl. 2, fig. 4 (1885) (!). *Lithophyllum papillosum* Foslie in "Siboga Exp.," Mem. 61: 63, fig. 23 (*optime!*) (1904); *Melobesia Cystosirae* Hauck., loc. cit., 266, pl. 3, figs. 1, 2, 6 (1885); Adriatic Sea.

***Goniolithon alternans* (Lemoine) comb. nov.**

Lithophyllum alternans Lemoine, *Archiv. Mus. Paris*, 4: 64-66, figs. 22, 23, pl. fig. 3, pl. 3, fig. 9 (1929) (!); Galapagos Islands.

Goniolithon geometricum (Lemoine) comb. nov.

Lithophyllum (*Dermatolithon*) *geometricum* Lemoine in Boergesen, "Rhodophyc. Canary Islands," 17, fig. 17, pl. 1, fig. 4 (1929); Canary Islands.

Goniolithon preprototypum (Lemoine) comb. nov.

Lithophyllum preprototypum Lemoine, "Contrib. Etud. Corallin. fossil.," No. 3: 265, fig. 12 (1917); fossil on Ile de Martinique, W. I.

Goniolithon prototypum (Foslie) comb. nov.

Lithophyllum prototypum Foslie, "On Some Lithoth.," 18 (1897); Island of Santa Cruz, W. I. Possibly same as *G. Udoteae* Foslie (according to Lemoine).

Goniolithon tessellatum (Lemoine) comb. nov.

Lithophyllum (*Dermatolithon*) *tessellatum* Lemoine, *Archiv. Mus. Paris* 4: 68-70, figs. 26, 27, pl. 1, figs. 3, 6, pl. 4, fig. 7 (1929) (!); Galapagos Islands.

Goniolithon Udoteae Foslie

Foslie, "New Melobesieae," 21 (1901); Island of Santa Cruz, W. I., see also *G. prototypum* (Foslie).

Goniolithon lapidea (Foslie) comb. nov.

Mastophora (*Lithostrata*) *lapidea* Foslie, "Algol. Notis.," II, 27 (1906); *Lithoporella lapidea* (Foslie) Foslie, "Syst. Bemerk.," 59 (1909); Caspian Sea.

Species of *Goniolithon* (*verum!*) are frequent in elevated limestones, probably of the early Eocene period, in the Lau Islands between Fiji and Tonga in the Pacific Ocean. Studies are still in progress.

Neogoniolithon nom. nov.

Fronds from crustaceous to decidedly fruticulose and branched; hypothallus (basal or medullary) coaxial; perithallus of erect filaments, arising from the hypothallus; epithallus more or less distinct of flattened cells; heterocysts frequent or sparse, typically in short vertical rows; conceptacles of all three types, opening by a single pore; tetrasporangia uniformly (always?) distributed over the floor of the tetrasporangial conceptacle. Type species, *Lithothamnium Fosliei* Heydrich, *Ber. deutsch. bot. Gesell.*, 15, 1897, p. 58, for the crustaceous species and *Goniolithon frutescens* Foslie, "Calcareous algae from Funafuti," 9 (1900), for the fruticulose or branched species. A considerable and widely distributed genus.

The following large number of incompletely known series of forms seem properly to be referred here.

Species Crustaceae

Neogoniolithon accretum (Foslie et Howe) comb. nov.

Goniolithon accretum Foslie et Howe, "New American Corall. Algae," 131, pl. 85, fig. 2, pl. 91 (1906); Bahama Islands, W. I. (!).

Neogoniolithon Fosliei (Heydrich) comb. nov.

Lithothamnium Fosliei Heydrich, *Ber. deutsch. bot. Gesell.*, 15, 1897, 58 (p.p.), 410; Maldive and Laccadive Archipelagoes (!).

Neogoniolithon Hariotii (Foslie) comb. nov.

Goniolithon Hariotii Foslie, "Algol. Notis.," III, 13 (1907); Mangareva, in the Marquesas Archipelago (!).

Neogoniolithon megalocystum (Foslie) comb. nov.

Goniolithon megalocystum Foslie, "Siboga Exp.," Mon. 61: 48, fig. 20, pl. 9, figs. 8, 9 (1904); East Indies.

Neogoniolithon misakiense (Foslie) comb. nov.

Goniolithon misakiense Foslie, "New Lithoth.," 4 (1905); E. Japan.

Neogoniolithon myriocarpum (Foslie) comb. nov.

Lithothamnium myriocarpum Foslie, "On Some Lithoth.," 19 (1897); "*Goniolithon myriocarpon*" Foslie, "Siboga Exp.," Mon. 61: 45, pl. 9, figs. 6, 7 (1904); East Indies (!).

Neogoniolithon orthoblastum (Heydrich) comb. nov.

Lithothamnium orthoblastum Heydrich, *Ber. deutsch. bot. Gesell.*, 19: 403 (1901). *Goniolithon orthoblastum* M. A. Howe, "Calc. Alg. fr. Murray Isl., Austral.," 291, pl. 97, fig. 2, pl. 98, figs. 1, 2 (1918); Papua (!).

Neogoniolithon pacificum (Foslie) comb. nov.

Goniolithon pacificum Foslie, "Nye Kalkalger," 6 (1908); E. Japan.

Neogoniolithon solubile (Foslie et Howe) comb. nov.

Goniolithon solubile Foslie et Howe, in Foslie, "Algol. Notis.," IV, 21 (1907); Island of Jamaica, W. I. (!).

Neogoniolithon versabile (Foslie) comb. nov.

Goniolithon versabile Foslie, "Algol. Notis.," III, 15 (1907); Japan.

Species Valde Verrucosae aut ramosae**Neogoniolithon affine** (Foslie et Howe) comb. nov.

Goniolithon affine Foslie et Howe, in Foslie, "Algol. Notis.," IV, 22 (1907); Island of Culebra, W. I. *An Hydrolithon? aff. G. Boergesenii* Foslie?

Neogoniolithon assitum (Foslie) comb. nov.

Goniolithon assitum Foslie, "Algol. Notis.," IV, 23 (1907); Red Sea. (*An Hydrolithon? aut Porolithon?*)

Neogoniolithon brassica-florida (Harv.) comb. nov.

Melobesia brassica-florida Harvey, "Nereis Australis," 110 (1849); Algoa Bay, S. E. Africa.

Neogoniolithon finitimum (Foslie) comb. nov.

Goniolithon finitimum Foslie, "Nye Kalkalger," 8 (1908); S. E. Australia.

Neogoniolithon frutescens (Foslie) comb. nov.

Goniolithon frutescens Foslie, "Calc. Algae Funafuti," 9 (1900); "Siboga Exp.," Mon. 61: 53, 54, fig. 22, pl. 10, figs. 7-9 (1904); Indo-Pacific (!).

Neogoniolithon laccadivicum (Foslie) comb. nov.

Goniolithon laccadivicum Foslie, "Siboga Exp.," Mon. 61: 51, pl. 9, figs. 10-13 (1904); Indian Ocean (!).

Neogoniolithon mamillare (Harv.) comb. nov.

Melobesia mamillaris Harvey, "Nereis Australis," 109, pl. 41 (1849); Brazil.

Neogoniolithon mamillosum (Hauck.) comb. nov.

Lithothamnium mamillosum Hauck., "Meeresalgen," 23, pl. 3, fig. 3, pl. 5, fig. 1 (1885); Adriatic Sea.

Neogoniolithon Martellii (Sams.) comb. nov.

Goniolithon Martellii Sams., "Sopra due Alghe Calc. foss.," 241 (1914); Fossil, Albania.

Neogoniolithon Rhizophorae (Foslie et Howe) comb. nov.

Goniolithon Rhizophorae Foslie et Howe, "New Amer. Corall. Alg.," 130 (1906); Bahama Islands, W. I.

Neogoniolithon strictum (Foslie) comb. nov.

Goniolithon strictum Foslie, "New Melobes.," 14, (1901); M. A. Howe, *Jour. N. Y. Bot. Garden*, 6, n. 64, with figure (1905); Florida and W. I. (!).

Neogoniolithon spectabile (Foslie) comb. nov.

Goniolithon spectabile Foslie, "New Melobes.," 16 (1901); Bermuda Islands. *G. strictum* Foslie et *G. intermedium* Foslie valde affine?

Neogoniolithon trichotomum (Heydrich) comb. nov.

Lithothamnium trichotomum Heydrich, "Lith. Mus. Paris," 538 (1901); Gulf of California. *G. frutescens* Foslie valde affine? (!)

The species transferred above seem reasonably to be referred to the second genus *Goniolithon* of Foslie, as renamed, of necessity, and by us, *Neogoniolithon*. Of the majority not mentioned here, renewed study of the type material is necessary to determine whether they are true *Neogoniolithons* or possibly to be referred to *Hydrolithon* or even to *Lithophyllum* or *Porolithon*.

NEW OR LITTLE KNOWN CRUSTACEOUS CORALLINES OF
PACIFIC NORTH AMERICA

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Since 1895 and even from somewhat earlier, the senior author has been collecting and studying crustaceous Corallines, especially those from the Indo-Pacific area. Very early many of the specimens, particularly from the coasts of Pacific North America, were sent to M. Foslie at Trondhjem, Norway, the great authority on the groups, and were named and published upon by him. There exists, therefore, in the Herbarium of the University of California many duplicate types and other authentic material for the study of Pacific North American species as well as of adjacent areas.

The junior author prepared, as a Ph.D. thesis, a detailed account of the species of the coasts of Pacific North America, with illustrations, critical notes, as well as a general review of the subfamilies, of the genera, and species, ranging from Bering Straits to Panama. A copy of this thesis is deposited with the Library of the University of California, where it may be consulted.

The work has been continued by both authors and in the course of some years additional facts and modification of opinion have resulted. The following notes as to changes of nomenclature, of new species, and other such matters are detailed below.

1. **Lithothamnium giganteum** L. R. Mason sp. nov.

Frons crustacea, durissima, usque ad 6–8 mm. crassa, protuberantibus robustissimis, 10–15 mm. altis, 5–15 μ latis, apicibus quam basibus latioribus, munita, colore obscure cinerea, magnitudine 5 cm. 7 cm. et ultra; hypothallo tenue, ad superficiem totam lapillorum mediocrium non calcareorum, per superficiem totam inferam, arcte adherente, 1–2 usque ad 80 cellulis crasso, cellulis 6–20 μ longis, 6–8 μ latis, elongatis cum substrato parallelis; perithallo 6–7 mm. crasso, crebre stratoso (stratis magnitudine formaque parvarum cellularum differentibus), cellulis isodiametricis, 5–6 μ diam., aut verticaliter leviterque elongatis 6–14 μ altis et 5–8 μ latis; epithallo 1–3 stratorum cellularum, cellulis horizontaliter applanatis, 2–4 μ alt., 5–7 μ lat.; conceptaculis tetrasporangiiferis 440–475 latis, 90–210 μ altis, poris 50–60 apertis; tetrasporangiis 4-divisis; conceptaculis spermatangiiferis cystocarpiiferisque nondum visis.

Spec. typ. La Jolla, California, No. 1514a; leg. N. L. Gardner.

This is by far the coarsest *Lithothamnium* on the Pacific coast of North America, as yet discovered. In habit and size, it superficially resembles *Lithothamnium crassiusculum* (Foslie) comb. nov. (see next species) but is more massive, thicker, harder and with more regularly arranged branchlets (or warts) swollen above and narrowed to a stalk-like base.

2. **Lithothamnium crassiusculum** (Foslie) L. R. Mason comb. nov.

Lithothamnium rugosum f. *crassiuscula* Foslie, "New Melobesieae," 4 (1900) (p.p.); *L. pacificum* f. *crassiuscula* Foslie, "Algol. Notis.," II, 10 (1906) (p.p.); Foslie, "Mon. Lithoth." (ed. H. Printz), 44, pl. 4, fig. 18 (1929).

Foslie based his "*crassiuscula*" on two specimens (Setchell 1149 and 1496a, the first from White's Point, near San Pedro, the second from San Pedro, California), referring it at first (1901) under *Lithothamnium rugosum* Foslie (1900), a Fuegian species, but later under *L. pacificum* Foslie (1906). Careful study both on the shore and on type material has convinced us not only of the independence of both *L. rugosum* and *L. pacificum* of one another but also of the fact that the f. *crassiuscula* included two different species, of which we refer No. 1149 Setchell to the proposed *Lithothamnium crassiusculum* and No. 1496a Setchell to an entirely new species, *Lithothamnium aculeiferum*, to be described below. The two species are very unlike both in habit and in structure. *L. crassiusculum* has coarse pro-

tuberances irregularly distributed but frequent, resembling superficially *L. giganteum* in habit, while *L. aculeiferum* has irregularly and sparsely distributed excrescences which are low and pointed.

3. *Lithothamnium aculeiferum* L. R. Mason sp. nov.

Frons crustacea, 1–3 mm. crassa ad saxa arcte per totam superficiem inferam adherente vulgo lapillos parvos ab undis circumjactis prorsus circumdata, colore vivo roseo-purpureo siccitate purpureo-cinereo, excrescentibus numerosis angularibusque, humilibus, 1–1.5 mm. alt., basi 1–2.5 mm. lat., vulgo ad apicem aculam attenuatis; hypothallo aut tenuiter evolvato aut passim usque ad 150–200 μ alt., cellulis elongatis, 10–30 μ long., 6–10 μ alt., parallelis, et in angulis rectis perithallum faciendum assurgentibus, perithallo 1–2.5 mm. crasso, saepe per differentiam parvam cellularum magnitudinis stratoso, cellulis isodiametricis, lente elongatis, aut ad superficiem superam paralleliter applanatis, ad angulos rotundatis, 5–8 μ lat., 3–9 μ alt.; epithallo 1–3 cellulo-stratos, cellulis 5–7 μ lat., 1–3 μ alt., applanatissimis, vulgo multo contusis; conceptaculis sporangiiferis convexis, 95–160 μ alt., vulgo ad superficiem superam parallelis leviter applanatis, poris 15–40, uniuersisque circum 20 μ diam.; tetrasporangiis 90–150 μ alt., 60–75 μ lat., transverse 2-divisis, conceptaculis cystocarpiferis convexis, 425–500 μ diam.; conceptaculis spermatangiiferis nondum visis.

Lithothamnium rugosum f. *crassiuscula* Foslie, "New Melobesieae," 4 (1901) (p.p.); *L. pacificum* f. *crassiuscula* Foslie, "Algol. Notis.," II, 10 (1906) (p.p.).

The type specimen is No. 1496a, Setchell, collected near San Pedro, California, and especially that part of the collection preserved in the Herbarium of the University of California. It has also been collected at Pacific Grove, California (Maurice B. Nichols, Nos. 126, 181, in Herb. Univ. Calif.), and at La Jolla, California (Maurice B. Nichols, No. 382, in Herb. Univ. Calif.).

Foslie's description of his f. *crassiuscula* seems to have been drawn from Setchell No. 1149, which differs from Setchell No. 1496a in habit, structure, number of pores of the tetrasporangial conceptacle, and number of divisions in the tetrasporangium. The name, promoted to specific rank, is retained for, and restored to, No. 1149 and its like, while No. 1496a (and its like) has been made the type of *Lithothamnium aculeiferum*.

4. *Lithothamnium validum* Foslie nom. delend.

Foslie, "Algol. Notis.," II, 10 (1906); Setchell and Gardner, *Proc. Calif. Acad. Sci.*, ser. 4, 19: 197 (1930); *Lithoth. rugosum* f. *valida* Foslie, "New Melobesieae," 4 (1900) (omnibus p.p.).

A study of a portion of the type material (Herb. Farlow, No. X, collected near San Diego, by H. Hemphill) indicates that this species of Foslie is a composite and therefore the binomial is to be rejected. The internal structure described is that of a *Lithothamnium* but the external structure is that of a *Lithophyllum*. The latter, later described by Foslie as *Lithophyllum imitans* ("Algol. Notis.," VI, 13 (1909)), is a species fairly readily recognized by its coarseness, covered by many short obtuse branchlets and the glossy (or glazed) surface of both the horizontal crust and the branchlets.

5. ***Melobesia mediocris* (Foslie) comb. nov.**

Lithophyllum mediocris Foslie, "Algol. Notis.," III, 33 (1906); *Lithophyllum zostericolum* f. *mediocris* Foslie, "Five New calcareous algae," 5 (1900); *Lithothamnium mediocris* Foslie et Nichols, in Nichols, *Univ. Calif. Pub. Bot.*, 3: 341-348, pl. 9 (1908); *Melobesia amplexifrons* Farlow, *Proc. Amer. Acad.*, 12: 239 (1877) (non Harv.).

Since *Melobesia*, as restricted in accordance with the type species, *M. membranacea* (Esper) Lamouroux ("Nouv. Bull. Sci.," p. 1. *Soc. Philomat.*, V, 3: 186 (1812)) must take precedence of *Epilithon* Heydrich ("Melobesiae," 408 (1897)), it is necessary to make this new combination for our Californian plant. It, together with *Melobesia marginata* Setchell et Foslie represents the genus on our coasts.

6. ***Lithophyllum neofarlowii* nom. nov.**

Lithophyllum Farlowii Foslie, "New Melobesiae," 12 (Feb. 18, 1901) non *L. Farlowii* Heydrich, "Lithoth. Mus. Paris," in Engler's *Bot. Jahrb.*, 28: 532, pl. 1, fig. 6 (Jan. 12, 1901).

The proposing of the name *Lithophyllum Farlowii*, at almost the same time, by Foslie for a Californian species and by Heydrich for a Galapagos species, was seemingly settled when Heydrich incorrectly assumed the priority of Foslie's name and rechristened the species from the Galapagos Islands *L. claudescens* Heydrich (*Ber. deutsch. bot. Gesell.*, 19: 420 (1901)). A careful examination of the exact dates (as shown above) indicates, however, that the *L. Farlowii* of Heydrich has prior claim, that the *L. Farlowii* Foslie must receive a new name and, further, that the *L. claudescens* Heydrich must be relegated to synonymy.

7. ***Lithophyllum elegans* (Foslie) Foslie**

Hariot sent certain of the specimens collected by Diguët off La Paz, Mexico, to Foslie, retaining others for his own study. Foslie and Hariot each, independently, gave names to the specimens in their own possession.

Both authors published in 1895, Hariot in the *Journal de Botanique* (vol. 9, pp. 166, 167) and Foslie in the journal of the Norwegian Society ("New or Critical Lithothamnium," in *Det. Kongl. Norske Vidensk. Selsk. Skrifter*, No. 2). The *Lithophyllum elegans* Foslie and the *Lithophyllum margaritae* Hariot undoubtedly are synonymous, but which was first published? Foslie claims (*Det. Kongl. Norske Vidensk. Selsk. Skrifter* 1901, No. 2, p. 20) that *L. elegans* has priority.

8. *Lithophyllum whidbeyense* Foslie

Lithophyllum whidbeyense Foslie ("den. bot. Samling," 5 (1906)) seems to be the same as *L. vancouveriense* Foslie (loc. cit.), judging from our studies of the types and of other collections from the same region. Since the description of *L. whidbeyense* precedes that of *L. vancouveriense* on the same page, that name seems to have a slight precedence.

9. *Heteroderma Nicholsii* nom. nov.

Dermatolithon pustulatum f. *typica* Foslie, "Algol. Notis.," VI, 46 (1909) (p.p.), *Lithothamnium pustulatum* f. *australis* Foslie, "Remarks on Northern Lithoth.", 117, 128 (1905) (p.p.), M. B. Nichols, *Univ. Calif. Pub. Bot.*, 3: 356, pl. 10, figs. 4, 5, pl. 13, figs. 21-24 (1905)!, is to be referred to the genus *Heteroderma* as finally limited by Foslie (Syst. Bemerk., in "Algol. Notis.," VI, 56 (1909)). *Heteroderma* seems to be the best solution for referring the very considerable number of non-heterocysted species formerly referred to *Melobesia*. Since *Melobesia* Lamour. (1812) is to be restricted to species with soriform tetrasporangial conceptacles, the majority of the species referred to *Melobesia* seem to fit into *Heteroderma* where Foslie has placed them in his 1909 paper. The type species may be considered to be *Heteroderma subtilissimum* (Foslie) Foslie (loc. cit., 1909, p. 56) from Papua. The genus includes all oligostromatic and non-heterocysted species devoid of a palisade-like hypothallus (*Dermatolithon*), but with the tetrasporangial conceptacles opening by a single pore. The plants which both Nichols and Foslie referred to *Dermatolithon*, from La Jolla, California, are neither of the genus *Dermatolithon* nor of the species *D. pustulatum*. The hypothallus (see Nichols, loc. cit., pl. 13, figs. 21-24) is *not* made up of cells vertically elongated, and the tetrasporangia are regularly 4-parted. A new name is necessary and we dedicate the plant so well described and illustrated by Maurice Barstow Nichols to him, in token of appreciation of his pioneer work among these forms.

10. *Dermatolithon ascripticum* (Foslie) comb. nov.

Dermatolithon pustulatum f. *ascripticia* Foslie, "Algol. Notis.," III, 34 (1906); *Lithophyllum pustulatum* f. *ascripticia* Nichols, *Univ. Calif. Pub. Bot.*, 3: 354, pl. 10, figs. 2, 3, pl. 11, fig. 10, pl. 12, figs. 18-20 (1909).

This Californian species seems to be independent of both *Dermatolithon macrocarpum* and *D. pustulatum*; from the former it differs in having 4-parted tetrasporangia and from the latter in cell dimensions of the thallus. The type specimens were collected by Mr. Nichols at Santa Catalina Island, California, on *Gelidium pyramidale* N. L. Gardner.

11. ***Dermatolithon saxicolum*** (Lemoine) comb. nov.

Lithophyllum (*Dermatolithon*) *saxicolum* Lemoine, *Arch. Mus. d'hist. nat., Paris*, 4: 48, fig. 10 of p. 51 (1929). On stones from 9 meters, Cocos Island, Costa Rica.

As described and figured, this species seems to be related to *Dermatolithon dispar* (Foslie) Foslie, but saxicolous rather than epiphytic. No specimens are available for examination.

12. ***Hydrolithon Setchellii*** (Foslie) comb. nov.

Goniolithon Setchellii Foslie, "Rev. Syst. Survey Melobesieae," 16 (1900); *Lithothamnium Setchellii* Foslie, "On Some Lithoth.," 18 (1897).

The present species seems to fit better into *Hydrolithon* than into the *Neogoniolithon* (*Goniolithon* of Foslie 1904, non *Goniolithon* subg. *Eugoniolithon* Foslie, 1898). The general habit and structure are in some ways intermediate. The heterocysts are sparse and single, and the hypothallus varies from a single layer (in the main basal portions) to somewhat more complex (in the overlapping lobes). The perithallic cells are somewhat irregularly placed, but do not vary so much in size and arrangement as in *Hydrolithon Reinboldii* (Weber et Foslie) Weber et Foslie.

13. ***Litholepis fertilis*** (Lemoine) comb. nov.

Melobesia (*Litholepis*) *fertilis* Lemoine, *Arch. Mus. d'hist. nat., Paris*, 4: 49, fig. 12 on p. 51, pl. 1, fig. 5 (1899); Coiba Island, Panama.

Mme. Lemoine compares the structure to that of *L. mediterranea* Foslie, but considers that it is to be distinguished from all other *Litholepis* species by the size of the (empty) conceptacles (275–500 μ diam.).

Additional new combinations for species of the Pacific coast of North America are to be published elsewhere.

LINEAR PARTIAL DIFFERENTIAL EQUATIONS WITH ANALYTIC COEFFICIENTS

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This paper contains some general results on linear partial differential equations with analytic coefficients; in particular it is concerned with questions of *uniqueness* of solutions of the Cauchy problem, and with the *functional character* of the solutions.

1. *The Cauchy Problem.*—The general linear m th order equation for a function $u(x_1, \dots, x_n)$ is given by

$$L[u] = \sum_{i_1 + \dots + i_n \leq m} A_{i_1 \dots i_n}(x_1, \dots, x_n) \frac{\partial^{i_1 + \dots + i_n}}{\partial x_1^{i_1} \dots \partial x_n^{i_n}} u = B(x_1, \dots, x_n) \quad (1)$$

Only real variables will be admitted. The $A_{i_1 \dots i_n}$ and B shall be regular analytic functions of those real arguments, wherever considered.¹

Equation (1) defines at each point $P = (x_1, \dots, x_n)$ the "characteristic" directions, i.e., those having direction numbers $\alpha_1, \dots, \alpha_n$ satisfying

$$Q_P(\alpha_1, \dots, \alpha_n) = \sum_{i_1 + \dots + i_n = m} A_{i_1 \dots i_n}(x_1, \dots, x_n) \alpha_1^{i_1} \dots \alpha_n^{i_n} = 0 \quad (2)$$

The *Cauchy problem* consists in determining a solution of (1) for which on a hypersurface S in $x_1 \dots x_n$ -space the "initial values," i.e., the values of $u, \partial u / \partial N, \dots, \partial^{m-1} u / \partial N^{m-1}$ ($\partial / \partial N =$ normal derivative), are prescribed. The method of power series as applied by Cauchy, Kowalewski and Weierstrass permits to prove the following general theorem:

If S is the image of an open $(n - 1)$ -dimensional full sphere under a non-singular analytic transformation, and no normal of S has a characteristic direction, and if the initial values are regular analytic on S , then for every closed subset Σ of S there is a neighborhood of Σ in $x_1 \dots x_n$ -space, in which there exists and is uniquely determined a regular analytic solution of (1) taking the prescribed initial values on Σ .²

As soon as the restriction to analytic solutions or to analytic initial values is discarded, the behavior of the solutions of the Cauchy problem varies largely with the "type" to which equation (1) belongs; hardly any general results, applying to all types, are known.³ One of the properties of differential equations, to be examined here, concerns the degree to which solutions behave arbitrarily on any manifold in space. A class F of functions defined in a set R in $x_1 \dots x_n$ -space may be called *arbitrary*, if the following is true: If \bar{R} is any closed subset of R , and P any point of R outside

\bar{R} , then there are two functions of F that have the same values in all points of \bar{R} , but not in P . In this sense the class of all analytic functions defined in an open set R , or defined on any analytic manifold in $x_1 \dots x_n$ -space, is not arbitrary. On the other hand, the functions of class C^m , where m is any non-negative integer, form an arbitrary class. Similarly arbitrariness can be defined for classes of systems of functions.

It is possible to obtain all solutions of class C^m of (1) by solving certain Cauchy problems with *analytic* initial values for the *adjoint* equation of (1), and by using the *completeness* of the set of analytic functions. This idea was used originally by E. Holmgren⁴ for the proof of certain "local" uniqueness theorems. We shall make fuller use of that idea here, chiefly proving uniqueness theorems not restricted to sufficiently small neighborhoods; those only will enable us to make conclusions about the functional behavior of the solutions of (1).

2. *The Fundamental Identity.*—As the main tool we derive an identity of the type of Green's identity for equation (1). Let S_λ denote a family of hypersurfaces given by an equation

$$\lambda = \lambda(x_1, \dots, x_n) = \text{const.}$$

to be specified later. We define "derived" differential operators $L^{(k)}[u]$ (with respect to λ) to the given operator L by the formulas

$$L^{(0)}[u] = L[u], \quad L^{(k+1)}[u] = \lambda L^{(k)}[u] - L^{(k)}[\lambda u]$$

Then

$$L^{(m)}[u] = (-1)^m m! \cdot Q_P \left(\frac{\partial \lambda}{\partial x_1}, \dots, \frac{\partial \lambda}{\partial x_n} \right) \cdot u, \quad L^{(m+1)}[u] = 0 \quad (3)$$

Let the "adjoint" operator $\bar{L}[u]$ be defined in the usual manner. An invariant element of surface $d\omega$ may be defined by

$$d\omega = \frac{dS_\lambda}{\sqrt{\sum_i \left(\frac{\partial \lambda}{\partial x_i} \right)^2}} = \frac{dx_1 \dots dx_n}{d\lambda}$$

where dS_λ is the ordinary element of surface of S_λ . The fundamental identity in question then takes the form

$$\int_{S_\lambda} u \bar{L}[w] d\omega = \sum_{k=0}^m \frac{1}{k!} \frac{d^k}{d\lambda^k} \int_{S_\lambda} w L^{(k)}[u] d\omega \quad (4)$$

This identity is invariant under arbitrary coördinate transformations. It can be proved for all "sufficiently regular" families of *closed* surfaces S_λ . It will be used here, however, for cases, where the S_λ are *open* surfaces with a *common boundary*. More exactly (4) is proved for the case that

(a) the S_λ are given by the relations

$$\frac{x_1}{1 - \sum_{i=2}^n x_i^2} = \lambda = \text{const.}, \quad \mu = 1 - \sum_{i=2}^n x_i^2 \geq 0 \quad (5)$$

(b) u is of class C^m , and

(c) w is of the form $\mu^{m-1} \varphi(\lambda, x_2, \dots, x_n)$, where λ and μ denote the functions of x_1, \dots, x_n defined in (5), and φ is of class C^m in λ, x_2, \dots, x_n .

3. *Uniqueness Theorems.*—In applying (4) we do not follow the usual procedure of choosing for w a solution of $\bar{L}[w] = 0$ with a *suitable singularity*, which will yield the value of u at one point. Instead we take for w a solution of the ordinary Cauchy problem

$$\bar{L}[w] = \bar{L}[(1 - \sum_{i=2}^n x_i^2)^{m-1} \varphi] = 0$$

$$\left(\frac{\partial^k \varphi}{\partial \lambda^k} \right)_{\lambda=\epsilon} = \begin{cases} 0 & \text{for } k = 0, \dots, m-2 \\ v(x_2, \dots, x_n) & \text{for } k = m-1 \end{cases}$$

According to Cauchy-Kowalewski this Cauchy problem for φ or w will have a solution, if S_ϵ has no characteristic normals, and if v is regular analytic. The solution w can then be shown to be an analytic function of $x_2, \dots, x_n, \lambda, \epsilon$ regular for

$$\sum_{i=2}^n x_i^2 \leq 1, \quad |\lambda - \lambda_0| \leq \delta, \quad |\epsilon - \lambda_0| \leq \delta$$

with a certain $\delta > 0$. By integration with respect to λ from λ_0 to ϵ (4) goes over into the following identity, valid for $|\epsilon - \lambda_0| < \delta$:

$$\int_{\lambda_0}^{\epsilon} d\lambda \int_{S_\lambda} w \cdot B(x_1, \dots, x_n) d\omega + \sum_{k=1}^m \frac{1}{k!} \left(\frac{d^{k-1}}{d\lambda^{k-1}} \int_{S_\lambda} w L^{(k)}[u] d\omega \right)_{\lambda=\lambda_0} =$$

$$\frac{1}{m!} \int_{S_\epsilon} \frac{\partial^{m-1} \varphi}{\partial \lambda^{m-1}} \mu^{m-1} L^{(m)}[u] d\omega = (-1)^m \int_{S_\epsilon} \mu^{m-1} Q_P(\alpha_1, \dots, \alpha_n) \cdot v \cdot u d\omega \quad (6)$$

where the $\alpha_i = \frac{\partial \lambda}{\partial x_i}$ are direction numbers of the normal of S_ϵ at the point

$P = (x_1, \dots, x_n)$ of S_ϵ . Now the left hand member of (6) depends analytically on ϵ ; hence, if u is a solution of (1) of class C^m and $v(x_2, \dots, x_n)$ is regular, the expression

$$I_v(\epsilon) = \int_{S_\epsilon} \mu^{m-1} Q_P(\alpha_1, \dots, \alpha_n) \cdot v \cdot u d\omega$$

is a regular analytic function of ϵ in a neighborhood of $\epsilon = \lambda_0$, provided S_{λ_0} has no characteristic normals. Furthermore applying (6) to $\lambda_0 = 0$, we

see that $I_\epsilon(\epsilon)$ for all sufficiently small ϵ is expressible in terms of u and its derivatives of order $\leq m - 1$ on S_0 , provided S_0 is free of characteristic normals. Moreover, it follows from the Fundamental Lemma of the Calculus of Variations that for a fixed ϵ , u is uniquely determined in all points of S_ϵ , if $I_\epsilon(\epsilon)$ is known for all analytic functions v .

Using all these facts one concludes that the initial values of u , $\partial u / \partial N$, \dots , $\partial^{m-1} u / \partial N^{m-1}$ on S_0 uniquely determine u on all S_ϵ , for which none of the surfaces S_λ with $0 \leq \lambda \leq \epsilon$ has a characteristic normal. A closer analysis shows that it is sufficient to assume here u to be of class C^{m-1} in the closed set covered by the S_λ with $0 \leq \lambda \leq \epsilon$, and to be of class C^m in all interior points of that set. Again, because of the invariance of the characteristic equation (2), the same statement holds, if the hypersurfaces S_λ for $0 \leq \lambda \leq \epsilon$, instead of being given by relations (5), can be transformed into the hypersurfaces (5) by a non-singular analytic transformation.

The resulting uniqueness theorem for the Cauchy problem for all equations (1) with analytic coefficients is very general, due to the wide choice left for the S_λ . It can roughly be formulated as follows: *The initial values on an analytic hypersurface S_0 uniquely determine u in all points which can be reached by deforming S_0 in such a way that its boundary is kept fixed and all surface elements with characteristic normals are avoided.* This generality is obtained, because the procedure followed here makes it unnecessary to know anything about the characteristic manifolds themselves in advance. In applying this uniqueness theorem to specific cases, it is only necessary to find out how far S_0 can be deformed without violating the restrictions; that will be different for different types of equations.

In this way we find, e.g., for any equation (1) with *constant* coefficients: If C is any *convex* $(n - 1)$ -dimensional set in the hyperplane $x_1 = 0$ containing the origin, then the initial values of u on C will uniquely determine u at the point $(1, 0, \dots, 0)$, if the polar reciprocal set C' of C formed with respect to the unit-sphere about the origin in the $x_2 \dots x_n$ -plane does not contain in its interior any point whose coördinates x_2, \dots, x_n satisfy $Q(1, x_2, \dots, x_n) = 0$. In the case of *normal-hyperbolic* equations with constant coefficients we may take for C the convex hull of the "wave-surface"; this is in agreement with uniqueness theorems obtained by K. Friedrichs and H. Lewy,⁵ and with the explicit solutions constructed by G. Herglotz.⁶

4. *Pseudo-Analytic Behavior along an Initial Hypersurface.*—From the general uniqueness theorem we can obtain information about the *impossibility* of the Cauchy problem for certain analytic hypersurfaces Σ . That situation will always arise, if there exists a 2-dimensional plane through the normal of Σ at a point P , which does not contain any direction characteristic at P . Let A be an n -dimensional neighborhood of P ; let s be that part of Σ lying in A , and let A' and A'' be the two *open* sets into which s may divide A . Then in the cases under consideration the system of functions

$u, \partial u / \partial N, \dots, \partial^{m-1} u / \partial N^{m-1}$ is *not arbitrary* on s , if u is assumed to be a solution of class C^m of (1) in A' and to be of class C^{m-1} in $A' + s$; there will indeed exist closed subsets s_0 and s_1 of s without common points, such that the values of the m initial functions in s_0 already uniquely determine the values of u in s_1 , as a consequence of the general uniqueness theorem.

Two points are to be observed in this connection: First, that the impossibility of the Cauchy problem is proved *locally*, nothing being assumed about the existence of u outside a neighborhood A of P . Second, that only the *whole system* of initial functions behaves in a non-arbitrary way; u alone might very well be arbitrary on the boundary manifold Σ .

With the help of the geometric test given, it can be seen that the Cauchy data cannot be prescribed arbitrarily on a "surface element" Σ in the following general cases:

- (a) when (1) is an elliptic equation and Σ any analytic surface element,
- (b) when (1) is a 2nd order equation, which is neither parabolic nor normal-hyperbolic, and Σ is any analytic surface element,
- (c) when (1) is a 2nd order normal-hyperbolic equation, and Σ not "space-like," and analytic.⁷

5. *Pseudo-Analytic Behavior along Interior Manifolds.*—The example of the potential equation suggests that the functional behavior of a solution u of (1) in *interior* points may be much more restricted than in boundary points. Such restricted behavior is indeed made evident by a study of the integrals of u over lower-dimensional manifolds in $x_1 \dots x_n$ -space.

For this purpose we prove the fundamental identity (4) for the more complicated family of hypersurfaces S_λ given by

$$\sum_{\alpha=1}^s [x_\alpha - (1 - \sum_{a=s+1}^n x_a^2)(c_\alpha - cb_\alpha + \lambda b_\alpha)]^2 = \lambda^2(1 - \sum_{a=s+1}^n x_a^2)^2$$

where s is a fixed integer with $0 < s \leq n$, and the c_α, b_α, c are constants subject to $\sum_{\alpha=1}^s b_\alpha^2 < 1$. Then the S_λ for $\lambda > 0$ all have a common $(n - s - 1)$ -dimensional boundary given by

$$\sum_{a=s+1}^n x_a^2 = 1, x_1 = \dots = x_s = 0$$

and approach for $\lambda, c, c_1, \dots, c_s$ converging toward 0, the $(n - s)$ -dimensional manifold

$$\sum_{a=s+1}^n x_a^2 \leq 1, x_1 = \dots = x_s = 0 \quad (7)$$

One concludes similarly as before, that for a regular analytic function $v(x_1, \dots, x_n)$ the expression

$$\int_{S_\lambda} u \cdot v \, d\omega \quad (8)$$

is a regular analytic function of $\lambda, c, b_1, \dots, b_s$ for sufficiently small positive λ and sufficiently small c, c_1, \dots, c_s , provided no normal of the manifold (7) has a characteristic direction. The following lemma is then applied: If the integrals of a continuous function $F(y_1, \dots, y_s)$ over spheres in $y_1 \dots y_s$ -space depend analytically on center and radius of those spheres, then F itself is a regular analytic function. This lemma permits to conclude from the analyticity of expressions (8) the following theorem:

If $M_{\lambda_1 \dots \lambda_s}$ denotes a family of $(n - s)$ -dimensional manifolds in $x_1 \dots x_n$ -space, which by a non-singular analytic transformation can be transformed into the manifolds.

$$\begin{cases} x_\alpha = \lambda_\alpha (1 - \sum_{a=s+1}^n x_a^2) & \text{for } \alpha = 1, \dots, s \\ \sum_{a=s+1}^n x_a^2 \leq 1 \end{cases}$$

and if dm denotes any regular analytic element of mass on $M_{\lambda_1 \dots \lambda_s}$, then

$$\int_{M_{\lambda_1 \dots \lambda_s}} u \, dm$$

for a solution u of (1) of class C^m in a neighborhood of $M_{\lambda_1 \dots \lambda_s}$ is a regular analytic function of $\lambda_1, \dots, \lambda_s$, as long as no normal of the manifold $M_{\lambda_1 \dots \lambda_s}$ has a characteristic direction.

This theorem may be considered as a generalization to arbitrary equations (1) of the known theorem (which here is included as the special case $s = n$), that if (1) is an elliptic equation, then $u(x_1, \dots, x_n)$ itself is a regular analytic function.⁸

From the analyticity of expressions (9) we can again conclude that the solution u is not an arbitrary function on certain manifolds. Let P be a point of an r -dimensional analytic manifold M in $x_1 \dots x_n$ -space. If there is an $(n - r + 1)$ -dimensional linear space, which contains all normals of M at P , but does not contain any direction characteristic at P , then the solutions of (1), which are of class C^m in an n -dimensional neighborhood of P , do not form an arbitrary class of functions on M itself in a neighborhood of P . (Here already the function u alone behaves in a non-arbitrary way.⁹)

¹ That is, shall be representable by power series locally.

² See Courant-Hilbert, *Methoden der mathematischen Physik*, Vol. II, pp. 39, et seq.; Hadamard, *Lectures on Cauchy's Problem*, Chap. I.

³ See the discussion by Hadamard, loc. cit., Chap. II.

⁴ Öfversigt af kongl. Vetenskaps-Akademiens Förhandlingar, pp. 91-103 (1901); Holmgren considers systems of linear 1st order equations with analytic coefficients for functions of two variables.

⁵ *Math. Annalen*, 98, 192-204 (1928); also *Math. Annalen*, 104, 325-339 (1931).

⁶ *Abh. Math. Sem. Hamburg. Univ.*, **6** (1928); *Ber. d. sächs. Akad.*, **78** (1926) and **80** (1928). See also Courant-Hilbert, loc. cit., pp. 455, *et seq.*

⁷ See on necessary or sufficient conditions for the Cauchy problem:

For the potential equation: Hadamard, loc. cit., p. 31, sec. 15 bis.

For the wave equation on time-like manifolds: Hadamard, loc. cit., pp. 349-357.

For hyperbolic equations with 3 independent variables: E. W. Titt, *Ann. Math.*, **40**, 862-891 (1939).

For a certain ultra-hyperbolic equation: G. Owens, *Duke Math. Jour.*, **9**, 271-282 (1942).

⁸ See Holmgren, loc. cit., 1901, pp. 437-456. S. Bernstein, *Math. Annalen*, **59**, 20-76 (1904). T. Rado, *Math. Zeit.*, **25**, 514-589 (1926). H. Lewy, *Math. Annalen*, **101**, 609-619 (1929); *Trans. Am. Math. Soc.*, **37**, 417, *et seq.* (1935). E. Hopf, *Math. Zeit.*, **34**, 194-233 (1931).

⁹ Special known instances of non-arbitrary behavior in interior points are: In the case of ultra-hyperbolic equations with constant coefficients: Courant-Hilbert, loc. cit., pp. 427-430; in the case of time-like manifolds for the "equation of Darboux": F. John, *Math. Annalen*, **113**, 541-559 (1935).

GROUPS CONTAINING A PRIME NUMBER OF CONJUGATE SUBGROUPS

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While there are many groups which have the property that each of them contains one and only one invariant proper subgroup there is no group which contains one and only one non-variant subgroup since a non-invariant subgroup is transformed under the group into another such subgroup of this group. The smallest number of non-invariant subgroups of a group is therefore two and when a group contains exactly two non-invariant subgroups they are necessarily conjugate under the group. Similarly when a group contains exactly three non-invariant subgroups they must all be conjugate under the group but when a group contains more than three non-invariant subgroups they may appear in more than one set of conjugates under the group. For instance, the group of the square contains four non-invariant subgroups which appear in two sets of conjugates under the group. It should be noted that a non-invariant subgroup of a group is necessarily a proper subgroup of it.

It is not difficult to construct an infinite system of groups of order p^m , p being any prime number, which has the property that every group contained therein involves exactly p non-invariant subgroups and that these subgroups constitute a single set of conjugates under the group. To construct such a system we may start with the cyclic group of order

p^{m-1} , $m > 3$, and extend this group by an operator of order p which transforms this cyclic group into itself and gives rise to a commutator of order, p . This extended group of order p^m contains $p + 1$ subgroups of order p , including the commutator subgroups of this order, which constitutes the central of the group. The remaining subgroups of order p are the p conjugate subgroups under the group of order p^m , which is conformal with the abelian group of order p^m and of type 1, $m - 1$. Every operator of this group whose order exceeds p generates the commutator subgroup of the group and every subgroup which contains such an operator is invariant.

When p is odd it is only necessary to assume that $m > 2$ in the preceding paragraph since the group of order p^3 will then also satisfy the condition that it contains exactly p non-invariant subgroups which constitute a single set of conjugate subgroups under the group, but when $p = 2$ there result two corresponding groups of order 8. One of these is the octic group and contains four non-invariant subgroups of order 2 which are conjugate in pairs, as was noted above. The other is the quaternion group, which involves no non-invariant subgroup but is also non-abelian. We shall prove in what follows that when $p = 2$ the given infinite system of groups includes all the groups which separately contain two and only two non-invariant subgroups but when p is odd there are other infinite systems composed of groups which separately involve exactly p conjugate subgroups.

Suppose now that the group G contains exactly two non-invariant subgroups. As each of these two subgroups is invariant under half the operators of G there must be a subgroup H of index 2 under G which involves this subgroup. The subgroup H must also contain the other non-invariant subgroup of G since this subgroup is also transformed into itself by exactly half of the operators of G and the operators of G which do not transform into itself the first of the two given subgroups must transform this subgroup into the second of these two subgroups and hence they do not transform this second subgroup into itself. That is, *every group which contains two and only two non-invariant subgroups contains a subgroup of index 2 which involves these two subgroups invariantly while each of the remaining operators of the group transforms these two subgroups into each other.*

Since all the subgroups of H are invariant under H it results that H is either abelian or Hamiltonian. It cannot contain more than two subgroups which are invariant under G because G is supposed to contain two and only two non-invariant subgroups. Hence it results that when H is Hamiltonian its order is a power of 2 and the order of G is twice the order of H . It is known that a Hamiltonian group whose order is a power of 2 is the direct product of the quaternion group and the abelian group of order 2^m and of type 1^m and that it contains a characteristic subgroup of order 2^{m+1} which is abelian and of type 1^{m+1} and involves all the operators of order 2 contained in this Hamiltonian group. Hence m could not exceed

zero. As it is known that a group of order 16 does not contain two and only two non-invariant subgroups it results that H is abelian whenever G contains two and only two non-invariant subgroups.

The operators of G which are not in H must include at least one whose order is a power of 2 since H is a subgroup of index 2 under G . Hence H cannot involve a Sylow subgroup of odd order since all the subgroups of G which are not contained in H are invariant under G . It therefore results that the order of G is a power of 2. As H involves at least two subgroups of the same order it cannot be cyclic. It cannot have more than two invariants since not more than two of its subgroups are invariant under G and G contains only two non-invariant subgroups. For the same reason one of the independent generators of H must be of order 2 and each of the operators of G which are not in H generates the same subgroup of order 2. The two non-invariant subgroups found in G are therefore of order 2 and all the operators of G which are not in H are of order 2^{m-1} , if the order of H is 2^{m-1} . It therefore follows that G belongs to the infinite system of groups noted above and there results the following theorem: *If a group contains two and only two non-invariant subgroups it is of order 2^m and it is conformal with the abelian group of type 1, $m - 1$. There is one and only one such group for every value of $m > 3$ and its commutator subgroup is of order 2.*

Suppose that a group G contains p conjugate subgroups, p being a prime number, and that all of its other subgroups are invariant. These conjugate subgroups are transformed under G according to a permutation group of degree p which has no more than p subgroups. Such a group involves only one subgroup of order p , since the subgroups of order p in this permutation group are Sylow subgroups, and if there were more than one such Sylow subgroup in it there would be at least $p + 1$. It therefore results that if a group contains a set of p conjugate subgroups while all of its other subgroups are invariant it must be either isomorphic with a permutation of degree p which contains an invariant subgroup of order p or it contains an invariant subgroup of index p which includes all of its non-invariant subgroups as invariant subgroups. In particular, *if a group contains a prime number of conjugate subgroups while all of its other subgroups are invariant then either no two of these subgroups transform each other into themselves or every two of these subgroups transform each other into themselves.*

When every two of the p conjugate subgroups of G transform each other into themselves, then G contains an invariant subgroup H of index p which involves these p subgroups invariantly and contains no non-invariant subgroup. Hence this invariant subgroup is either abelian or Hamiltonian. It is easy to prove that it could not be Hamiltonian since every Hamiltonian group contains a characteristic subgroup of order 2 and hence could not involve exactly an odd prime number of non-invariant subgroups. It therefore results that H is abelian and that the order of H is a power of p since

the operators of G which are not in H include at least one whose order is a power of p . The facts that H cannot have more than two invariants, cannot be cyclic and when it has two invariants, one of them is p , follow exactly in the same way when p is an odd prime number as when $p = 2$. It therefore results that whenever every two of the p non-invariant conjugate subgroups transform each other into themselves then G belongs to the infinite system of groups considered in the second paragraph of this article.

It remains to consider the case when no two of the conjugate subgroups of G transform each other into themselves and hence G involves an invariant subgroup which gives rise to a quotient group which is simply isomorphic with a non-cyclic transitive group of degree p involving an invariant subgroup of order p . In the simplest case this transitive group is the symmetric group of degree 3 and all the possible groups can be obtained by establishing a dimidiation between this group and the cyclic group of order 2^m . Moreover, each of the groups thus obtained satisfies the condition that it contains three and only three conjugate subgroups such that no two of these subgroups transform each other into themselves. This completes the determination of all the group which involve three and only three non-invariant subgroups.

Every transitive group of degree p which involves an invariant subgroup of order p is known to contain p and only p non-invariant subgroups whenever its order is pq , q being a prime divisor of $p - 1$, and only then. By establishing a $p, q^{\alpha-1}$ isomorphism between this transitive group and the cyclic group of order q^{α} , $\alpha > 1$, there results a group of order pq^{α} which satisfies the condition that it contains p conjugate subgroups and that each of its other subgroups is invariant. This completes the determination of all the groups which separately contain a prime number of conjugate subgroups while all of their other subgroups are invariant.

NEW CHARACTERIZATIONS OF SEGMENTS AND ARCS

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I. The writer has for some time been interested in studying the metric and topological properties of various classes of metric spaces when these spaces are assumed to be *free from equilateral k -tuples*. It has been shown, for example, that a complete, convex, externally convex metric space M (containing at least two points) is metrically a straight line if and only if M

does not possess an equilateral triple.¹ Indeed, any metric space containing a line L and a point not belonging to L^* has an equilateral triple.

On the topological side, one inquires what topological properties a space must possess in order that it may be homeomorphic to a space with prescribed metric properties. The metrization problem is subsumed under this general class of problems as a "first case."

In this note two new theorems belonging to such a *Programme* are announced and their proofs briefly sketched. One of the theorems was conjectured by the writer in a paper now in press, and the other is closely related to the metric characterization of the line cited above.²

II. Characterization Theorems.

THEOREM 1. *A compact convex metric space M of at least two points is congruent with a line segment if and only if M is free from equilateral triples.*

Proof. If M is congruent with a line segment, then obviously M is free from equilateral triples. Assuming M free from such triples, we note that M contains two points p, q at maximum distance and (since M is convex) a segment (p, q) (i.e., a set of points congruent with a line segment) with end-points p and q . We suppose M has a point r not belonging to (p, q) and show this implies the existence of an equilateral triple.

Case 1. *There exists an interior point s of (p, q) such that a segment with end-points r and s contains neither of the subsegments $(p, s), (s, q)$ of (p, q) .*

Then (r, s) has a point r^* , with arbitrarily small positive distance from (p, q) , such that a point r_0^* of (p, q) nearest r^* is an interior point of (p, q) . The method of a previous paper may now be applied to prove the existence of two points on (p, q) which, together with r^* , form an equilateral triple.³

Case 2. *The situation described in Case 1 does not hold.*

It follows that a point s of (p, q) with maximum distance d from r is interior to (p, q) and hence each segment (r, s) contains either (p, s) or (q, s) . Selecting the labeling so that $(p, s) \subset (r, s)$, we have $rp + ps = rs = d$. It is easy to show that for each interior point t of the subsegment (q, s) , any segment (r, t) contains the subsegment (q, t) and hence, letting t approach s , it follows that q is interior to a segment (r, q, s) of length d .

Clearly $pq = ps + sq = d - pr + d - rq = 2d - (pr + rq)$. Since pq is a maximum distance in M , we have $pq \geq d$ and hence $d \geq pr + rq$. On the other hand, $pq \leq pr + rq \leq d$, and so $pq = d$ and r is between p and q . It is easily shown that the segments (r, p, s) and (r, q, s) have no points other than r and s in common.

Let, now, t and v be points of (r, p, s) and (r, q, s) , respectively, such that $rt = rv = 2d/3$. We observe that $v \in (r, q)$ readily implies $t \in (p, s)$.⁴ If v and t are end-points of a segment containing neither (p, t) nor (t, q) the presence of an equilateral triple with v as one vertex follows as in Case 1.⁵ Suppose, then, that any segment joining v to t contains either (t, q) or (p, t) . If such a segment contains (t, q) , then $vt = ts + sv = 2d/3$. The other

possibility is easily seen to lead to a contradiction, and the theorem is proved.

THEOREM 2. *A metric Peano continuum without an equilateral triple is an arc.*

Proof. It suffices to show that the continuum contains neither a simple closed curve nor a tripod (i.e., three arcs which have only a single point—an end-point of each—in common). Since such figures are one-dimensional continua, they may, according to a theorem of Beer,⁶ be *convexified* (i.e., a topologically equivalent metric may be introduced in terms of which the figures are metrically convex). Applying Theorem 1 to the convexified figures it is seen that they surely contain equilateral triples (else they would be segments and the original figures arcs). A close examination of Beer's procedure leads one to conclude that if equilateral triples are present in the convexified simple closed curve or tripod, then such triples occur in the original metric, and the theorem is established.

¹ Blumenthal, L. M., and Robinson, C. V., "A New Characterization of the Straight Line," *Reports of a Mathematical Colloquium*, 2nd Ser., Issue 2, 25-28 (1940).

² Blumenthal, L. M., "Some Imbedding Theorems and Characterization Problems of Distance Geometry," *Bull. Amer. Math. Soc.* (in press).

³ See reference 1.

⁴ If $v \in (q, s)$ then $t \in (r, p)$ or $t \in (p, s)$. The first case differs only in labeling from the one discussed, while in the second case we have immediately $vt = 2d/3$.

⁵ The cases (a) $p = t$ and (b) $v = q$ offer no difficulties.

⁶ Beer, G., "Beweis des Satzes, dass jede im kleinen zusammenhängende Kurve konvex metrisiert werden kann," *Fundamenta Mathematicae*, **31**, 281-320 (1938).

SOME PRELIMINARY STUDIES ON THE MECHANISM OF VIRUS MULTIPLICATION*

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The most distinctive characteristic of the process of virus multiplication is its dependence on the integrity and active growth of the infected host cells (e.g., Findlay and MacCallum¹). Except for some studies on the enzymic complement of vaccinia virus,^{2, 3} nothing is known concerning the mechanism of this dependence. The following is a preliminary account of some studies designed to investigate this problem.

In order to approach this problem, it was necessary first of all to establish criteria which would distinguish the general metabolic reactions of the

cell associated with normal cellular activity from those reactions specifically involved in virus multiplication. This is extremely difficult to do in the cells of higher animals and plants. Bacteriophage, the virus of bacteria, was found to be suitable material for such an approach, for the end result of the overall, normal metabolic activity of the host cells is multiplication, which can be measured very simply in culture. Hence, it is possible, by interfering with or enhancing the level of metabolism of the cell infected with virus, to determine concomitantly the extent of the effect, if any, on the multiplication of the virus.

Relation between Cell Growth and Virus Multiplication.—The viruses studied were two strains of anti-*Escherichia coli* bacteriophage, P₁⁴ and γ .⁵

An extensive study was made of virus P₁ in regard to the influence of various substrates on virus and cell growth.^{6, 7, 8} A number of amino-acids and closely related compounds were found capable of supporting both cell and virus growth. In general, whenever cell growth occurred concomitant virus multiplication took place. Two exceptions to this were found, namely, in the case of glycine and glycine anhydride (2,4-diketopiperazine). These substances, in low concentrations, were insufficient by themselves for cell multiplication, but supported quite considerable virus multiplication (table 1).

CONCENTRATION RANGE OF GLYCINE AND GLYCINE ANHYDRIDE IN WHICH P₁ MULTIPLICATION OCCURRED, BUT NO B₁ GROWTH (FOR 135 MINUTES)

GLYCINE	GLYCINE ANHYDRIDE
$5 \times 10^{-4} M$ to $5 \times 10^{-3} M$	$2 \times 10^{-4} M$ to $3 \times 10^{-3} M$

NOTE: At $5 \times 10^{-3} M$ glycine and $3 \times 10^{-3} M$ glycine anhydride, the virus multiplication (step-size in 60 minutes) was practically optimum.

In another series of studies designed to investigate the influence of metabolic inhibitors on virus multiplication, it was found that in a certain range of concentrations of sodium arsenite, the growth of virus γ on its host in a

MULTIPLICATION OF VIRUS γ AND HOST CELLS (60 MINUTES) IN AN ASPARAGINE-GLUCOSE-PHOSPHATE MEDIUM WITH VARIOUS CONCENTRATIONS OF SODIUM ARSENITE ADDED 8 MINUTES AFTER INFECTION (HOST CELLS PREVIOUSLY GROWN IN THE SYNTHETIC MEDIUM)

[illegible]

highly purified asparagine-glucose-phosphate medium⁹ was completely inhibited, while the host cells multiplied at their normal rate (the host cells being in the logarithmic phase) (table 2).

To sum up, then, it is possible to separate the process of virus multiplication from the growth of the host cells. Under certain conditions, cell growth may proceed unhindered while virus multiplication is inhibited completely. Under other conditions virus multiplication may proceed at a normal rate while cell divisions are inhibited.

Effect of Metabolic Inhibitors and Some Substrates on Virus Multiplication.—In order to understand the kind of metabolic reactions which are associated with virus multiplication in the cell, a survey was made of the effect of various metabolic poisons on virus growth when no cell multiplication occurred, as in certain concentrations of glycine and glycine anhydride (table 3).

TABLE 3

INFLUENCE OF METABOLIC POISONS ON VIRUS P₁ MULTIPLICATION IN 8.7×10^{-4} M GLYCINE ANHYDRIDE

INHIBITOR	CONC. (M)	% INHIBITION OF VIRUS MULTIPLICA- TION (100 MIN.)
Sodium cyanide	2×10^{-3}	100
Iodoacetic acid	5×10^{-4}	100
Sodium arsenite	6×10^{-4}	100
Phlorizin	2×10^{-3}	0
2,4-Dinitrophenol	5×10^{-4}	100
p-Aminophenol	1×10^{-3}	100
Arsenic pentaoxide	5×10^{-4}	40
Urethane	1×10^{-3}	0
Sodium fluoride	2.4×10^{-3}	0
Sodium sulfite	4×10^{-4}	0
Malonic acid	1×10^{-3}	0
Sodium pyrophosphate	2.5×10^{-4}	0

Hence, cyanide, iodoacetate, arsenite, dinitrophenol, and p-aminophenol appear to inhibit reactions associated rather closely with virus P₁ multiplication.

The influence of a large number of substrates on virus P₁ propagation in 8.7×10^{-4} M glycine anhydride was also studied, under conditions where there was no considerable effect on the cell multiplication. The effect of some of the more effective substrates is summarized in table 4.

These results suggest that the multiplication of virus P₁ is associated with certain specific cellular reactions known to be involved in the respiratory nexus of the cell. More detailed studies with these and other metabolic poisons and substrates may yield more precise data as to the kind of reactions involved in this and other viruses. Such studies are of prime importance for the investigation of possible therapeutic measures for virus diseases.

TABLE 4

INFLUENCE OF SOME SUBSTRATES ON VIRUS P₁ MULTIPLICATION IN 8.7×10^{-4} M GLYCINE ANHYDRIDE

(Bacterial multiplication not appreciably influenced in all cases except yeast nucleic acid)

SUBSTRATE	CONC. (M)	% INCREASE IN P ₁ (100 MIN.)
α -Glycerophosphoric acid	6×10^{-4}	150
Glucose-6-phosphate	4×10^{-4}	50
Adenosine triphosphate	2×10^{-4}	85
Coenzyme I	1×10^{-4}	80
Adenylic acid	3×10^{-4}	26
(Guanylic acid	3×10^{-4}	0)
Yeast nucleic acid	Less than 1×10^{-5}	350
Oxaloacetic acid	7.5×10^{-4}	200
α -Ketoglutaric acid	7×10^{-4}	260
Succinic acid	8.5×10^{-4}	95
Fumaric acid	8.5×10^{-4}	56
Calcium hydrogen malate	5.5×10^{-4}	145
Uric acid	5×10^{-4}	100
Xanthine	6.5×10^{-4}	15

The Arsenite Effect.—It is evident from table 2 that concentrations of 7.5×10^{-5} M and over of sodium arsenite are sufficient to inhibit the multiplication of virus γ in an asparagine medium (100% inhibition at 1.5×10^{-5} M), but bacterial growth cannot be inhibited with less than about 5×10^{-5} M. This is true when arsenite is added to virus-infected cells 8 minutes after infection, but not when the arsenite is added sooner (table 5).

TABLE 5

INFLUENCE OF PREVIOUS CONTACT OF HOST CELLS WITH ARSENITE ON γ MULTIPLICATION IN AN ASPARAGINE-GLUCOSE MEDIUM

HISTORY OF HOST CELLS	γ MULTIPLICATION IN ARSENITE CONCS. OF (M)						
	2.5×10^{-5}	5×10^{-5}	1×10^{-4}	1.5×10^{-4}	2.5×10^{-4}	5×10^{-4}	
24 Hours in 2.5×10^{-5} M sodium arsenite before infection	94	98	..	92	96	92	..
In 1.5×10^{-5} M arsenite 2 minutes before infection with γ and for 8 minutes thereafter	59	..	59	59	45	17	1

NOTE: Differences in virus multiplication figures for 0 arsenite in the two series are due to the difference in the number of infected cells in the two experiments, and not to the effect of the previous history.

From table 5 (cf. also table 2) it is evident that the longer the cells are exposed to arsenite previous to infection with virus the less effect the same

concentration of arsenite will have on virus γ multiplication. However, previous exposure of the cells to arsenite does not appear to influence the ability of the cells to support γ multiplication in the absence of arsenite. (In all cases cited there appeared to be no influence on the bacterial growth.)

Additional experiments designed to investigate the nature of the arsenite effect indicated that the arsenite effect is *instantaneous*, as was suggested by the fact that previous exposure of the cells to arsenite for 2 to 10 minutes altered the influence of various low concentrations on γ multiplication. Thus, when arsenite was added to a mixture of growing cells and virus at different times during the latent period of virus growth, there was a definite inhibition of virus multiplication. Even when arsenite ($1.5 \times 10^{-5} M$ and $2.5 \times 10^{-5} M$) was added 20 minutes after infection (one minute before the end of the latent period of γ multiplication under these conditions, after which there is a rapid release of virus) there is about 60% inhibition of virus growth.

On the other hand, the arsenite inhibition of virus growth can be relieved almost instantly on its removal. When arsenite ($2.5 \times 10^{-5} M$) was added to cells 2 minutes prior to infection with virus γ , and replaced with fresh asparagine-glucose medium containing no arsenite at 25 minutes after infection, the step-size value⁴ for virus growth was almost the same as if no arsenite had been present to begin with.

These experiments need extension, but certain conclusions are already evident. Arsenite appears to have a somewhat more specific effect on the virus than on the cell growth. The inhibiting action of arsenite on virus growth is readily relieved by replacing the arsenite with fresh medium lacking arsenite. The arsenite effect is extremely rapid, for it is capable of stopping the release of most of the virus by the cells at the end of the latent period of virus multiplication. Finally, the cell appears to be capable of "adapting" itself to the presence of arsenite, at least as far as the processes which lead to virus propagation are concerned.

The importance of understanding the mechanism of the arsenite effect cannot be underestimated. It is intimately concerned with the problem of the identity of the metabolic reactions involved in virus multiplication, some of which at least may not be involved in cell growth. The identification of the reactions influenced by arsenite may be studied by classical biochemical methods. As far as is known, no such studies have been made with bacteria. Krebs¹⁰ and Borsook and Dubnoff¹¹ studied the effect of arsenite on metabolic systems in sliced mammalian kidney tissue, and concluded that arsenite is an inhibitor of the reactions involved in the oxidation of α -keto acids. Whether this is true for bacteria remains to be seen.

Summary.—The processes of virus multiplication and cell growth can be separated experimentally in the two bacterial viruses and their corre-

sponding hosts studied. Under conditions in which virus multiplication proceeds in the absence of cell growth, it was found that certain specific metabolic poisons (cyanide, iodoacetate, arsenite, dinitrophenol, *p*-aminophenol) would inhibit virus growth, while others did not. Certain substances known to be involved either as intermediates or as coenzymes in intermediary carbohydrate metabolism in the cell were shown to enhance virus multiplication in the absence of bacterial growth.

Finally, arsenite was found to be a specific inhibitor of the multiplication of virus γ . The study of this inhibition may give valuable results concerning the nature of the reactions involved in virus multiplication.

* Part of this work was performed during the tenure of a National Research Council Fellowship in the Medical Sciences. Present address: Department of Bacteriology, Loyola University, School of Medicine, Chicago, Ill.

¹ Findlay, G. M., and MacCallum, F. O., *Brit. Jour. Exp. Path.*, **21**, 173 (1940).

² Parker, R. F., and Smythe, C. V., *Jour. Exp. Med.*, **65**, 109 (1937).

³ Hoagland, C. L., *et al.*, *Ibid.*, **72**, 139 (1940); **71**, 737 (1940); **74**, 769 (1941); **74**, 133 (1941).

⁴ Active on a strain of *Escherichia coli* (B₁) originally studied by Ellis, E. L., and Delbruck, M., *Jour. Gen. Physiol.*, **22**, 365 (1939).

⁵ From the original PC of Kalmanson, G. M., and Bronfenbrenner, J., *Ibid.*, **23**, 203 (1939), active on another strain of *Es. coli*.

⁶ Ellis, E. L., and Spizizen, J., *Science*, **92**, 91 (1940).

⁷ Spizizen, J., *Jour. Biol. Chem.*, **140**, cxxiv (1941).

⁸ Spizizen, J., Thesis, California Institute of Technology, 1942.

⁹ So-called "chemically pure" asparagine may often contain significant amounts of thiamin and biotin, as well as traces of other biologically active substances. Hence in this work asparagine was repeatedly crystallized before use. An ammonia-glucose-phosphate medium was also found to be sufficient for host and virus growth when a magnesium or calcium salt was present.

¹⁰ Krebs, H. A., *Zeit. physiol. chem.*, **217**, 191 (1933).

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¹¹ Borsook, H., and Dubnoff, J. W., *Jour. Biol. Chem.*, **141**, 717 (1941).

THE DETERMINATION OF L.D.50 AND ITS SAMPLING ERROR IN BIO-ASSAY, II

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If a bio-assay¹ were made at three dilutions D_1 , $D_2 = aD_1$, $D_3 = a^2D_1$ and if the probabilities P_1 , P_2 , P_3 , assumed to be in increasing sequence in the universe but with fluctuations due to sampling, were observed, the growth curve

$$P = 1/2 + 1/2 \tanh \alpha(x - \gamma) \quad (1)$$

containing the two parameters α , γ could be fitted to the three observed proportions P_1 , P_2 , P_3 , corresponding to the three values of the logarithmic dose x_1 , x_2 , x_3 , which are in arithmetical progression with the difference $c = \log a$. The natural method to use would be R. A. Fisher's method of maximum likelihood.² We shall use the middle dilution as a point of reference $x_2 = x$ with the other two at $x - c$, $x + c$; then the probability of the observations s_1 , s_2 , s_3 in the three series is

$$\frac{(n!)^3 P_1^{s_1} P_2^{s_2} P_3^{s_3} Q_1^{n-s_1} Q_2^{n-s_2} Q_3^{n-s_3}}{s_1! s_2! s_3! (n-s_1)! (n-s_2)! (n-s_3)!},$$

where $Q_i = 1 - P_i$ and P_i is taken from (1); and its natural logarithm, the likelihood, is (except for a constant which does not depend on α or γ)

$$L = \sum s_i \log [1 + \tanh \alpha(x_i - \gamma)] + \sum (n - s_i) \log [1 - \tanh \alpha(x_i - \gamma)]. \quad (2)$$

The derivatives with respect to α and γ are

$$\frac{\partial L}{\partial \gamma} = -n\alpha \sum (2P_i - 1) + n\alpha \sum \tanh \alpha(x_i - \gamma), \quad (3)$$

$$\frac{\partial L}{\partial \alpha} = n \sum (2P_i - 1)(x_i - \gamma) - n \sum (x_i - \gamma) \tanh \alpha(x_i - \gamma). \quad (4)$$

Placing x_1 , x_2 , x_3 equal to $x - c$, x , $x + c$ and setting the derivatives equal to zero we have

$$\sum (2P_i - 1) = \tanh \alpha(x - c - \gamma) + \tanh \alpha(x - \gamma) + \tanh \alpha(x + c - \gamma), \quad (5)$$

$$(2P_1 - 1)(x - c - \gamma) + (2P_2 - 1)(x - \gamma) + (2P_3 - 1)(x + c - \gamma) = (x - c - \gamma) \tanh \alpha(x - c - \gamma) + (x - \gamma) \tanh \alpha(x - \gamma) + (x + c - \gamma) \tanh \alpha(x + c - \gamma).$$

The first equation may be multiplied by $x - \gamma$ and subtracted from the second; then

$$2(P_3 - P_1) = -\tanh \alpha(x - c - \gamma) + \tanh \alpha(x + c - \gamma). \quad (6)$$

We have to solve (5) and (6) for α and γ .

Let $A = \sum (2P_i - 1)$, $B = P_3 - P_1$, and expand the tanh functions setting $X = \tanh \alpha(x - \gamma)$, $C = \tanh \alpha c$. Then

$$A = \frac{X - C}{1 - XC} + X + \frac{X + C}{1 + XC} = \frac{3X - 2XC^2 - X^3C^2}{1 - X^2C^2}, \quad (7)$$

$$2B = -\frac{X-C}{1-XC} + \frac{X+C}{1+XC} = \frac{2C(1-X^2)}{1-X^2C^2}. \quad (8)$$

Or

$$C^2[X^3 - AX^2 + 2X] = 3X - A, \quad C^2BX^2 + C(1 - X^2) = B. \quad (9)$$

Eliminating C , the quantity X is determined from the cubic

$$3X^3 - 4AX^2 + (6 + A^2 - 4B^2)X - 2A = 0. \quad (10)$$

Given the numerical values of A and B from the observations, the cubic may be solved numerically by any method of approximation, but for most cases the result

$$X = \frac{4A}{9} - \frac{b}{a} + \frac{729}{a} \left(\frac{b}{a} \right)^3, \quad \begin{cases} a = 1458 - 189A^2 - 972B^2 \\ b = 162A - 20A^3 - 432AB^2 \end{cases} \quad (11)$$

is sufficient, and often the third term may be omitted. Then³

$$\alpha = \frac{1}{c} \tanh^{-1} \frac{2B}{X^2 - AX + 2}, \quad \gamma = x - \frac{c \tanh^{-1} X}{\tanh^{-1} \frac{2B}{X^2 - AX + 2}}. \quad (12)$$

For example, if $n = 20$ and $s_1 = 8$, $s_2 = 15$, $s_3 = 20$ in $1/2$ dilutions so that $c = 0.6931$ (using natural logarithms), $P_1 = 0.40$, $P_2 = 0.75$, $P_3 = 1.00$, $A = 1.30$, $B = 0.60$,

$$X = 4 \frac{1.30}{9} - \frac{-35.516}{788.67} + \frac{729}{788.67} \left(\frac{-35.516}{788.67} \right)^3 = 0.57778 + 0.04503 - 0.00008 = 0.62273,$$

$$\alpha = \frac{1}{c} \tanh^{-1} (0.7603), \quad \gamma = x - c \frac{\tanh^{-1} 0.6227}{\tanh^{-1} 0.7603}.$$

As good tables of the \tanh^{-1} function may not be at hand one may use the logarithmic equivalent

$$\tanh^{-1} = \frac{1}{2} \log_e \frac{1+x}{1-x}, \quad \alpha = \frac{1}{2c} \log_e \frac{1.7603}{0.2397}, \quad \gamma = x - c \frac{\log_e \frac{1.6227}{0.3773}}{\log_e \frac{1.7603}{0.2397}}$$

With $c = 0.6931$ we have $\gamma = x - 0.5071$, $\alpha = 1.438$. For many purposes the table of hyperbolic functions in B. O. Peirce's *Short Table of Integrals*, page 126 (1929) may be adequate; natural logarithms may be replaced by logarithms to the base 10 if x , c , α , γ are all expressed logarithmically to that base.

R. A. Fisher's formula for the standard errors of the parameters calls for the mean values of the second derivatives of L . There are

$$\frac{\partial^2 L}{\partial \gamma^2} = -n\alpha^2 \Sigma \operatorname{sech}^2 \alpha(x_i - \gamma) = -4n\alpha^2 \Sigma P_i Q_i,$$

$$\frac{\partial^2 L}{\partial \alpha^2} = -n \Sigma (x_i - \gamma)^2 \operatorname{sech}^2 \alpha(x_i - \gamma) = -4n \Sigma P_i Q_i (x_i - \gamma)^2,$$

$$\frac{\partial^2 L}{\partial \alpha \partial \gamma} = n\alpha \Sigma (x_i - \gamma) \operatorname{sech}^2 \alpha(x_i - \gamma) = 4n\alpha \Sigma P_i Q_i (x_i - \gamma)$$

where the values of P_i are taken from the fitted curve rather than from the sample. The Hessian

$$H = \begin{vmatrix} \frac{\partial^2 L}{\partial \gamma^2} & \frac{\partial^2 L}{\partial \alpha \partial \gamma} \\ \frac{\partial^2 L}{\partial \alpha \partial \gamma} & \frac{\partial^2 L}{\partial \alpha^2} \end{vmatrix} = \frac{\partial^2 L}{\partial \gamma^2} \frac{\partial^2 L}{\partial \alpha^2} - \left(\frac{\partial^2 L}{\partial \alpha \partial \gamma} \right)^2$$

is then formed and the standard errors are⁴

$$\sigma_\alpha^2 = -\frac{1}{H} \frac{\partial^2 L}{\partial \alpha^2}, \quad \sigma_\gamma^2 = -\frac{1}{H} \frac{\partial^2 L}{\partial \gamma^2}.$$

The correlation between sampling variations in γ and α is by Fisher's formula

$$r_{\alpha\gamma} = \frac{\frac{\partial^2 L}{\partial \alpha \partial \gamma}}{\sqrt{\frac{\partial^2 L}{\partial \alpha^2} \frac{\partial^2 L}{\partial \gamma^2}}}.$$

It is not often that one wishes this value, but there is an interesting connection between the standard error that would be found for γ if α were considered as known and the standard error found on the basis of no such assumption which may be exhibited in terms of r . For if α were taken as known

$$\sigma_\gamma^2 = (-\partial^2 L / \partial \gamma^2)^{-1}$$

and

$$\frac{\sigma_\gamma^2, \alpha \text{ known}}{\sigma_\gamma^2, \alpha \text{ unknown}} = \frac{H}{\frac{\partial^2 L}{\partial \gamma^2} \frac{\partial^2 L}{\partial \alpha^2}} = 1 - r^2.$$

The value of r is zero when $\Sigma P_i Q_i (x_i - \gamma) = 0$, and this will nearly vanish whenever the probabilities on the fitted line are nearly symmetric with

respect to the middle dilution⁵ (the middle dilution being near the 50% point).

According to the conventions of Least Squares, the weight of an observation is proportional to the reciprocal of the square of its standard error. If we could consider the determination of γ on the assumption that α is known (or that there happens to be no correlation between α and γ), we should have

$$\frac{1}{\sigma_{\gamma}^2} = -\frac{\partial^2 L}{\partial \gamma^2} = 4n\alpha^2 \sum P_i Q_i.$$

Hence the weight contributed to the determination of γ by each point on the curve is proportional to $4P_i Q_i$ and will be relatively small for those values of P which are near 0 or 1. Thus the addition of dilutions of very high or of very low strengths would contribute very little to the accuracy of the determination⁶ of γ . If, however, we should enquire about the determination of α on the hypothesis that γ was known (or not correlated with α) we should have

$$\frac{1}{\sigma_{\alpha}^2} = -\frac{\partial^2 L}{\partial \alpha^2} = \frac{4n}{\alpha^2} \sum P_i Q_i [\tanh^{-1}(2P_i - 1)]^2$$

and it is clear that values near $P_i = 1/2$ will contribute very little. As a matter of fact those values of P in the neighborhood of 0.08 or 0.92 have the greatest weight. It is thus indicated that if we are assaying a biological to determine L.D.50 and we may assume from previous experience that the homogeneity, α , is known, we should aim to use such dilutions as will bring most of the observations near $P = 1/2$, but that if we are working with α unknown it is necessary to have a sufficient spread of P from the different dilutions so that α may be adequately determined.⁷

As $A = 2P_1 + 2P_2 + 2P_3 - 3$ and $B = P_3 - P_1$ are the combinations of the P 's which enter into the determination of the parameters α , γ of the curve, the same curve will be obtained for any set of P 's which give the same values of A and B , and in particular the values of P_1 , P_2 , P_3 on the fitted curve will be connected by the same relations. Hence in comparing the observed values with the fitted values we shall have

$$P_{1 \text{ obs}} - P_{1 \text{ fitted}} = P_{3 \text{ obs}} - P_{3 \text{ fitted}} = -1/2(P_{2 \text{ obs}} - P_{2 \text{ fitted}}).$$

In view of these relations we may easily set up all the different sets of s_i and $n - s_i$ which will lead to the same values of α and γ . For example if $s_1 = 8$, $s_2 = 15$, $s_3 = 20$ we have the three possibilities

s	8	15	20		7	17	19		6	19	18
$n - s$	12	5	0		13	3	1		14	1	2

The fitted values of s are 7.39, 16.23, 19.39. Any one of these tables may be compared with the fitted solution by a χ^2 -test, but the test is not very good because of the small numbers in some of the six cells. If we are safe in using the general rule we have proposed for a cellular universe,⁸ we should write as the relative probabilities of the three possibilities

1	1	1
8 ! 15 ! 20 ! 12 ! 5 ! 0'	7 ! 17 ! 19 ! 13 ! 3 ! 11'	6 ! 19 ! 18 ! 14 ! 1 ! 12 !

which are as 663, 600, 50 with a sum of 1313 and give actual relative probabilities of 0.505, 0.457, 0.038, which would mean that the third set of values would be a bad fit judged at the 0.05 level of significance.⁹ Actually with the variability of experimental animals one could hardly expect that the variation in different experiments should not exceed that due to pure chance.

¹ See these PROCEEDINGS, 29, 79-85 (1943).

² Fisher, R. A., *Phil. Trans. Roy Soc. (London)*, A222, 309-368 (1932). In this paper Fisher applies the method (p. 363) to another type of dilution series. Tables for the case of three series at tenth dilutions with $n = 10$ for each are given by Halvorson and Ziegler in their *Quantitative Bacteriology*, Burgess Publishing Co., Minneapolis, 1938. R. D. Gordon, these PROCEEDINGS, 24, 212-215 (1938) has indicated that the results of Halvorson and Ziegler are not in accord with careful experimental findings and has suggested the replacement of Fisher's method of maximum likelihood by the older method of inverse probability, and has given a longer treatment in *Biometrika*, 31, 167-180 (1939) followed by a comment by E. Pearson. If elaborate investigation shows that the principle of maximum likelihood applied to a certain hypothetical formulation of the problem of determining pollution by dilution experiments does not give the right results, either the principle or the hypothetical formulation will have to be given up or modified; but for our present problem we shall accept the principle.

³ Since $\alpha(x - \gamma) = \tanh^{-1} X$, $\alpha = \frac{1}{c} \tanh^{-1} C$, and $C = \frac{2B}{X^2 - AX + 2}$ from (9).

⁴ These formulas will give the standard errors for any number of dilutions provided the summations are carried over all the dilutions and the curve has been fitted well enough by any manner so that P_i can be adequately determined from it. For the problem solved above in the text the values of the standard errors may be determined as follows: measuring x from the middle dilution as 0, $x_1 = -0.6931$, $x_2 = +0.6931$, $x_1 - \gamma = 0.1860$, $x_2 - \gamma = 0.5071$, $x_3 - \gamma = 1.2002$. On the fitted curve $4P_1Q_1 = \text{sech}^2 \alpha(x_1 - \gamma) = \text{sech}^2 (-0.2675) = 1 - \tanh^2 (-0.2675) = 1 - (0.2612)^2 = 0.9318$. Similarly $4P_2Q_2 = 0.6124$, $4P_3Q_3 = 0.1190$. $\partial^2 L / \partial \gamma^2 = -1.6632n\alpha^2$, $\partial^2 L / \partial \alpha^2 = -0.3611n$, $\partial^2 L / \partial \alpha \partial \gamma = 0.2800n\alpha$. Then $H = 0.5222n^2\alpha^2$, $\sigma_\alpha^2 = 3.185/n$ and for $n = 20$, $\sigma_\alpha^2 = 0.1592$, $\sigma_\gamma^2 = 0.6915/(n\alpha^2) = 0.3344/n = 0.01672$. We may therefore write $\gamma = -0.5071 \pm 0.1293$, $\alpha = 1.438 \pm 0.399$. If we should have a second sample of three dilutions with $P_1' = 0.05$, $P_2' = 0.30$, $P_3' = 0.75$ we should find $\gamma' = 0.3054 \pm 0.1190$, $\alpha' = 1.440 \pm 0.3652$. It is clear that $\alpha = \alpha'$ much more nearly than could be expected by sampling. The difference $\gamma' - \gamma = 0.8124 \pm 0.1757$, and the difference is therefore highly significant. The antilogarithm of 0.8124 is 2.25 and of 0.1757 = 1.19 which means that the ratio 2.25 of the strength of the first to that of the second is affected by a sampling factor of 1.19 or 0.84 which amounts to about 17 1/2% or 2.25 =

0.39. It may be noted that the 3-dilution problem here worked out corresponds to the 2-dilution problem of our previous note (with P and P' interchanged) where the result was 2.24 ± 0.49 . In this case the result of taking the two best P 's (not two corresponding P 's) from a set of three in each of two dilutions is not materially different from or much more poorly determined than the result coming from the somewhat more elaborate calculations using all three dilutions.

⁵ For the cases of the previous footnote $r = 0.361$, $r' = -0.201$; in both of which the value of r^2 is small.

⁶ Thus, in the illustration, $P_1 = 0.40$, $P_2 = 0.75$, $P_3 = 1.00$ as observed led to $P_1 = 0.37$, $P_2 = 0.81$, $P_3 = 0.97$ for the curve and these contributed respectively $4P_1Q_1 = 0.9318$, $4P_2Q_2 = 0.6124$, $4P_3Q_3 = 0.1190$ to $1/\sigma_\gamma^2$; if we had had an additional dilution giving a value $s_0 = 1$ or 2 and let us say $P_0 = 0.07$ on the average, its contribution would be 0.2604 , which would be more than that due to $s_3 = 20$, and so far as the determination of γ goes it would have been better to use this dilution than the one which led to $s_3 = 20$; but in respect to the determination of γ' for which the first observed point was $s_1' = 1$, the weaker dilution would add $s_0' = 0$ and P_0' on the curve would be about 0.0076 giving a value of $4P_0'Q_0' = 0.030$ which would add practically nothing to the determination. When two biologicals are of strengths as different as these two it might be well to use different sets of three dilutions in comparing them.

⁷ If we wished to determine only the difference between two values of L.D.50 in two biologicals with no desire to determine γ , γ' , α or α' we could set up the maximum likelihood solution for $d = \gamma' - \gamma$, using $\gamma_0 = \frac{1}{2}(\gamma + \gamma')$, d , α and α' as four variables. This would come back to the solution obtained by treating α , γ together and α' , γ' together and forming the difference $\gamma - \gamma'$ and its standard error. But if it were justifiable to assume that α , though unknown, were the same for both biologicals and only γ and γ' were different, we could set up the problem in terms of the three variables γ_0 , α , d . The solution would still be the same as for $\gamma - \gamma'$ provided the values of P_i and P_i' were such as to give no correlations between α and γ or α and γ' in the separate solutions and if the correlations were small the solution would not differ very much either for the difference itself or for its standard error; α would be better determined, but that would be of slight advantage.

⁸ These *PROCEEDINGS*, 28, 378-384, 384-390 (1942), with especial reference to pages 382 and 388.

⁹ The corresponding value of χ^2 is 6.2, indicating a probability level, with one degree of freedom, of 0.013 instead of 0.038. It may be noted that the mean values of any s is not the fitted value—thus, the mean value of s_1 is 7.47 instead of 7.39.

CONCERNING THE RELATION BETWEEN STRUCTURE AND
ACTION OF XANTHONES ON DEHYDROGENATIONS BY
FUSARIA*

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Communicated February 26, 1943

According to Schöpf,¹ the formation of natural substances in the living cell may be of three types. (a) The cell may have an enzymatic system designed for the highly specific synthesis of a certain substance. (b) The second type is the result of processes of general application performed by enzymes, e.g., hydrogenations, dehydrogenations, decarboxylation, etc. (c) Such cases as take place without the action of enzymes and are characterized by the formation in the growth of the cell of reactive organic substances, which upon contact in the cell yield isolable products, which are, in fact, chance products or intermediates in the synthesis of the former, when their further conversion within the cell proceeds slower than their synthesis.

In the course of enzyme studies² conducted at this laboratory with different fusaria, an attempt was made to approach the problem of the function of certain "waste materials," such as pigments in plant cells or in microorganisms. In an earlier investigation³ the observation was made that *Fusarium oxysporum* contains a pigment, which may be related to aurofusarin.⁴ Attempts to extract or to accumulate this pigment proved to be futile. Consequently, recourse was taken to *Fusarium graminearum* Schwabe (Fgra) which, when cultivated on certain media, develops with abundant deposition of a reddish orange pigment (rubrofusarin).

Recently it was observed⁵ that the addition of nicotinic acid (NA) makes it possible more readily to synthesize dehydrogenating enzymes abundantly present in fusaria. It seemed justifiable to investigate whether there exists a functional relation between the two structurally different groups of substances.

Enzyme Material Used.—*Fusarium lini* Bolley (FIB) No. 5140 from the Biologische Reichsanstalt, Berlin-Dahlem, through the courtesy of Dr. H. Wollenweber. Fgra No. A 36-1-VIII from the University of Minnesota, through the courtesy of Dr. C. J. Eide. Stock cultures of the species were maintained on the following nutrient medium:

20.00 g. glucose
1.00 g. potassium nitrate
1.50 g. potassium phosphate (primary)
0.75 g. magnesium sulfate, 7H₂O
1000 ml. water

and transferred at two-weeks intervals. The cultures were periodically examined to check their purity.

Cultural Conditions.—For the experiments with NA and the various xanthoncs, a salt solution consisting of the following:

- 5.00 g. potassium nitrate
- 5.00 g. potassium phosphate (primary)
- 0.75 g. magnesium sulfate, 7H₂O
- 1000 ml. water

was sterilized at 120°C. under 15 pounds' pressure for 15 minutes.

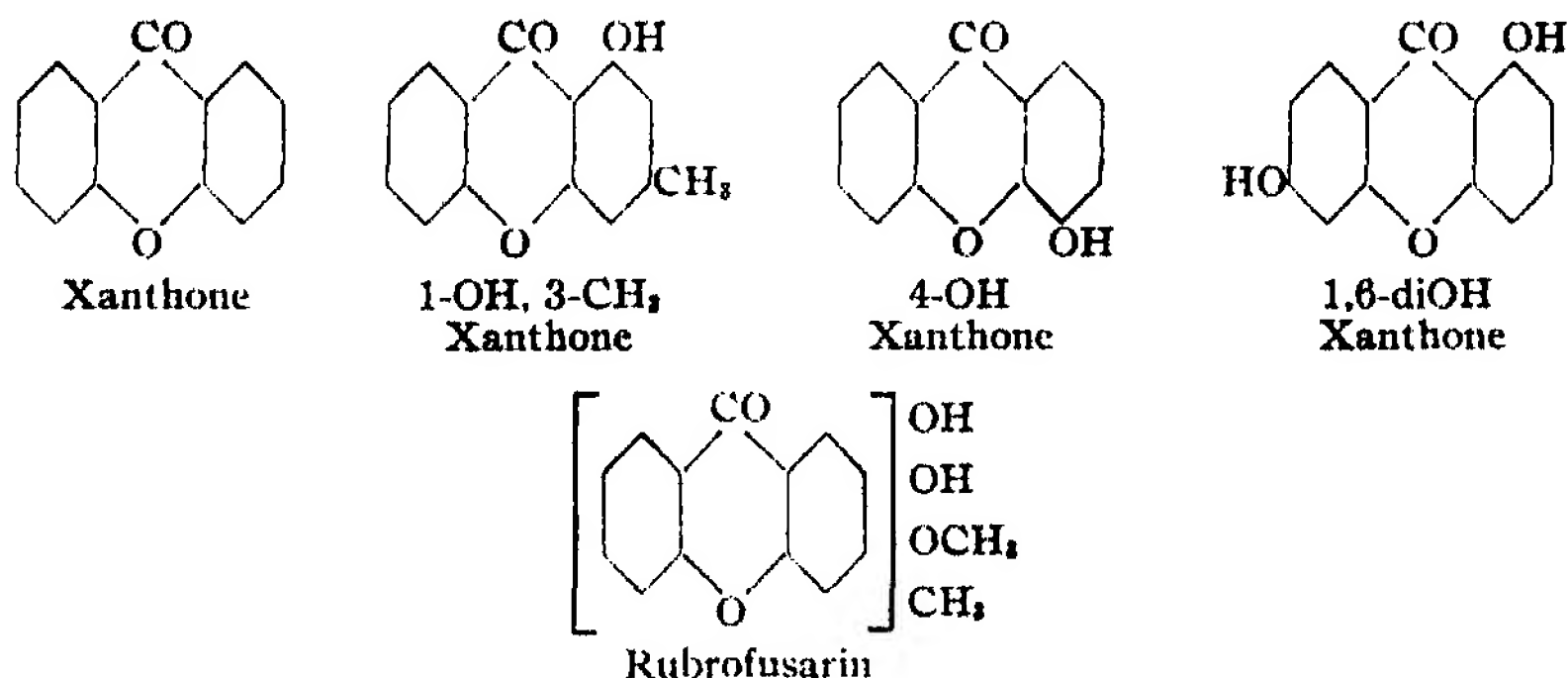
The isopropyl alcohol (Propol) was purified and the 81–81.7°C. fraction utilized as substrate, being added with sterile technique to the sterilized salt solution. In the case of NA, a previously sterilized water solution containing the required amount was added to the salt solution with sterile technique. With the various xanthoncs, the compound being investigated was first dissolved in Propol and then added to the salt solution. The concentration of Propol in all cases was approximately 0.4% and the initial pH 4.4. In all these experiments the FlB was grown in 125-ml. Erlenmeyer flasks containing 50 ml. of nutrient medium. Forty flasks were used for each series of experiments. Inoculation was by means of spores grown on the stock nutrient medium containing 2% agar.

For the production of the pigment a Raulin-Thom nutrient medium was used. A sterilincubator containing 14 Pyrex trays, each filled with 3 liters of nutrient medium, was used for the growth. The calculated amount of sterile 2 *N* sodium hydroxide was added later to change the pH to 8. Inoculations were by means of *Fgra* spores grown on the stock nutrient medium, containing 2% agar. After 3 weeks, the mats were removed, filtered, washed with water and dried in a vacuum oven at 40°C. The mats were then ground and exhaustively extracted[†] in a Soxhlet with petrol ether 40–60°C. Crude rubrofusarin separated upon cooling. This organism supplied us with 500 mg. of a crude composite pigment, obtained from 375 g. of dry mats. Purification was by means of sublimation and recrystallization. Chromatographic adsorption was also used as an alternative method for obtaining pure rubrofusarin, the analysis of which indicated that the pigment was a xanthone derivative, C₁₅H₁₂O₅ (see later).

Analytical Methods.—Four flasks were removed for each analysis and the combined filtrates analyzed in duplicate. The nutrient medium was filtered from the mycelium and 100 ml. of the filtrate was made alkaline with 10 ml. of 1 *N* sodium hydroxide and distilled in an all-glass apparatus. About 85 ml. of the distillate was collected and the volume brought up to 100 ml. Twenty-ml. aliquots of the distillate were used for determining Propol and acetone. Propol was analyzed by addition of the aliquot to a

mixture of 10 ml. of 0.4 *N* potassium dichromate and 15 ml. of concentrated sulfuric acid at room temperature. This was let stand 30 minutes and diluted with water to 400 ml. Fifteen ml. of 20% potassium iodide solution were then added and titrated with 0.1 *N* sodium thiosulfate to the starch end-point. Acetone determinations⁶ were carried out as heretofore. Mycelial weights were determined after 13 days by filtering, drying and weighing⁶ the mycelium from 5 flasks.

Discussion.—Starting with a Propol solution of the pigment, the effect of solutions upon the rate of dehydrogenation by FIB of the following substituted and basic xanthenes was investigated:



The table presented below shows the extent of effect observed on the rate of dehydrogenation of Propol and accumulation of acetone in the presence of given amounts of these compounds as compared with the magnitude of the rate of the same reaction effected by adding NA. Mat weight determinations at the end of each series of experiments complement the observations.

It can be seen, that in accordance with the structural formulae of our xanthenes, the following relations exist: xanthone (e.g., 8th day: 12.84%) and NA (in accordance with the concentrations: 1.99%, 2.69%, 4.13%, 5.69%) increase measurably the rate of dehydrogenation as compared with the blank. The three intermediates tend to show, in general, an increase (e.g., 8th day: 11.34%, 10.66%, 5.33%) in this rate to a distinctly lesser extent, and the natural pigment definitely retards the progress of dehydrogenation (e.g., 8th day: -3.01%) in accordance with the increasing number of hydroxyl groups and complexity of the xanthone molecule.

In evaluating the analytical data it has to be borne in mind that two dehydrogenations proceed parallel to one another. The dehydrogenation of the Propol is attended by that of the acetone formed. The latter reaction leads⁶ to formaldehyde via methanol as intermediary:

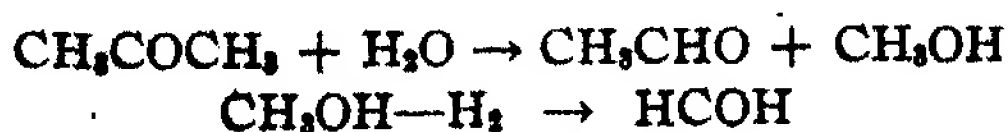


TABLE 1
DEHYDROGENATION OF ISOPROPYL ALCOHOL*

Concentrations of various xanthenes and nicotinic acid are expressed in γ per 1000 ml.

DAY	BLANK		XANTHONE 1000		1-OH 3-CH ₃ XANTHONE 1000		4-OH XANTHONE 1000		1,6-dioH XANTHONE 1000		RUBRO-PUSARIN 1000		BLANK		NA 500		NA 1000		NA 5000		NA 10,000	
	PRO-POL	ACE-TONE	PRO-POL	ACE-TONE	PRO-POL	ACE-TONE	PRO-POL	ACE-TONE	PRO-POL	ACE-TONE	PRO-POL	ACE-TONE	PRO-POL	ACE-TONE	PRO-POL	ACE-TONE	PRO-POL	ACE-TONE	PRO-POL	ACE-TONE	PRO-POL	ACE-TONE
0	180.0		181.6		182.7		186.0		183.0		180.0		183.5		187.5		187.0		189.0		185.0	
7	63.0	14.7	72.1	15.2	72.2	15.2	68.0	17.0	65.4	12.0	60.3	12.2	69.5	14.5	72.3	11.7	72.8	13.7	72.5	12.6	74.0	14.0
8	73.2		82.6		81.5		81.0		77.1		71.0		80.5		79.0		80.5		80.5		81.4	
9	86.3	17.3	91.6	19.3	90.6	21.0	94.1	18.7	88.9	17.2	80.3	14.2	88.8	16.8	89.0	15.8	85.0	16.0	93.2	17.3	90.7	15.3
10	106.0		110.3		112.2		110.0		104.3		96.3		101.7		103.3		104.1		98.5		104.3	
11	108.8	22.7	111.1	24.5	119.2	26.7	124.0	32.2	119.3	28.5	100.3	19.8	108.0	20.2	112.5	20.0	114.7	20.5	120.1	22.5	120.4	25.0
12	120.8		122.9		123.2		130.5		129.7		116.7		121.2		123.0		127.5		129.0		132.0	
13	131.8	33.8	139.8	32.6	138.2	29.0	134.3	35.5	144.0	28.1	126.8	32.9	130.5	25.8	133.5	24.3	135.8	28.0	137.2	28.5	139.8	26.3
MYCELIUM WEIGHT ON 13TH DAY																						
		26 mg.	29.4 mg.	35.0 mg.	39.9 mg.	41.7 mg.	35.5 mg.	31.7 mg.	29.3 mg.	35.4 mg.	32.4 mg.	42.5 mg.										

* Measured as amount in mgs. disappearing per 50 ml. of nutrient medium.

The extremely high rate of this reaction is recognizable towards the end and, consequently, the terminal Propol values appear to be higher in the case of extended experiments.

Although the increase in mat weight in the case of basic xanthone is only slight, the influence on the rate of dehydrogenation was greatest. In the case of the pigment, however, an approximately 40% increase of the final mat weight still was accompanied by a definite retardation of the rate of dehydrogenation.

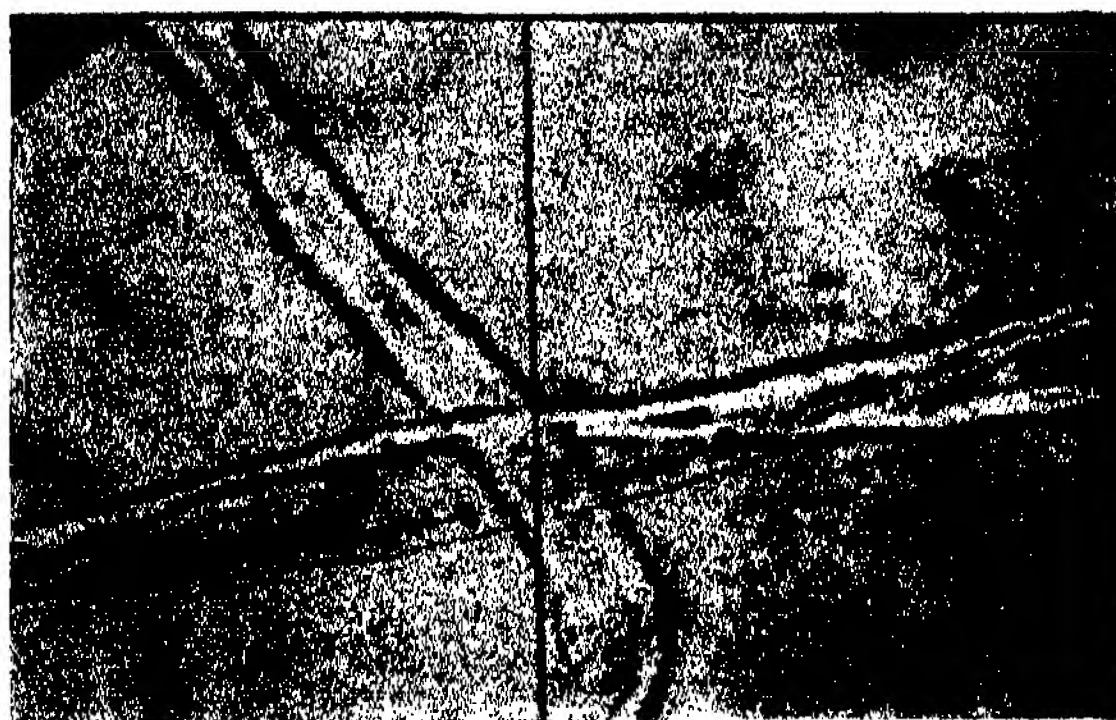


FIGURE 1

FIB grown on a Propol solution of xanthone (9th day, $\times 400$).



FIGURE 2

FIB grown on a Propol solution of rubrofusarin (9th day, $\times 400$).

Contrary to figure 1 above, the fungus in figure 2† indicates a visible deposition of the pigment excreted by Fgra, which is not utilized by FIB. This deposition is accompanied by enhanced growth.

These experiments seem to represent the first attempt to explain a possible mode of functioning of "waste products" in microorganisms.

Summary.—From the recorded values it is evident that the xanthoncs applied both augment and decrease the growth of FLB, accordingly giving rise to a higher or retarded rate of dehydrogenation of Propol, as compared with the values of the blank or with the effect of NA.

* This work was supported in part by a grant from the Rockefeller Foundation.

† About 100 g. of a mixture of fats were collected in the course of extraction which are being further investigated.

‡ The microphotographs were obtained through the courtesy of Dr. L. Yanowski.

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NOTICE. Owing to the preoccupation of members of the NATIONAL ACADEMY OF SCIENCES and of the NATIONAL RESEARCH COUNCIL with scientific aspects of the war effort, the manuscripts available for the March issue were so few that they were held over for this combined March-April number. It is not unlikely that from time to time similar combinations of numbers may be advisable, and it may not be possible always to give advance notice thereof.

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*MASTOPHORA AND THE MASTOPHOREAE: GENUS AND
SUBFAMILY OF CORALLINACEAE*

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Communicated March 24, 1943

The genus *Mastophora* was proposed and properly validated by Decaisne early in 1842, but, because of his arrangement of topics, it has been generally misunderstood by later writers, especially as to identity of its type species. Later in 1842, Decaisne in a separate paper reduced his genus under *Melobesia* and this has assisted in the confusion. The two papers of Decaisne, (1) "*Essais sur une classification des algues*" (*Ann. Sci. Nat., bot.*, ser. 2, 17: 297-380, pl. 14-17, 1842) and (2), "*Mémoires sur les Corallines*" (*Ann. Sci. Nat., bot.*, ser. 2, 18: 96-128, 1842) were reprinted (and repaged) as "*Thèses présentées et soutenues à la Faculté des Sciences de Paris, le 19 Decembre 1842,*" and were better known in this form to Harvey, Kuetzing and Areschoug, who shaped our knowledge of the Coralline Algae when these organisms were becoming recognized as plants rather than as being relegated to the animal kingdom. The inclusion of the two papers in one and with consecutive paging is partly responsible for misstatements occurring from 1842 onward.

On p. 69 of the reprint (p. 365 of vol. 17 of the *Ann. Sci. Nat., bot.*, ser. 2), Decaisne proposes *Mastophora* as a new genus, gives a short but distinctive Latin diagnosis, designates it as a marine alga of the habit of a *Lichen* or of a *Zonaria*, and names a type specimen: "*exsicc. no. 2232*" of "Cumming (or Cuming?) from Manilla." On pp. 63 and 84 of his reprint (pp. 359 and 380 of vol. 17, *Ann. Sci. Nat., bot.*, ser. 2, 1842) Decaisne gives an outline of the genera of his "*Corallineae*" and here as well as in his explanation of plate 17, fig. 11, he coins the binomial *Mastophora licheniformis* Decaisne, giving a thoroughly satisfactory figure of a branch of the plant, together with (under Fig. 11a) a vertical section through a tetrasporic conceptacle showing the single apical pore and the supporting distinctly monostromatic thallus. In consequence, it may be emphasized that *Mastophora* was provided by Decaisne with a Latin diagnosis, a type

species with a type specimen (*Mastophora licheniformis* Dec'ne and Cuming's Exss. No. 2232) and distinctive illustrations.

In his second paper, "Mémoire sur les Corallines" (pp. 85-116 of the reprint and pp. 96-128 of vol. 18 of *Ann. Sci. Nat., bot.*, ser. 2, 1842), Decaisne says (p. 127, p. 115 of the reprint) that *Melobesia* varies considerably in consistency and form, and that he has changed his point of view as to the importance of different modes of division of the fronds as well as of completeness, or lack of it, of adherence to the substratum. He therefore, in consequence of more profound study, reduces his *Mastophora* to a section of *Melobesia*. He names first *Melobesia* (*Mastophora*) *licheniformis* Decaisne (with *Zonaria rosea* C. Ag., non Lamour. as a synonym), second *M. Lamourouxii* Dec'ne (with *Padina rosea* Lamour. herb., *Dictyota rosea* Lamour. and *Peysonnelia* Dec'ne Pl. Arab., as synonyms) and, as a third species, describes *Melobesia tenuis* Dec'ne from the Sandwich Islands. It is to be noted that the three binomials of this second deliverance of Decaisne are *Melobesia* (*Mastophora*) *licheniformis*, *Melobesia* (*Mastophora*) *Lamourouxii* and *Melobesia* (*Mastophora*) *tenuis*, all new species of Decaisne, but practically never referred to under their proper binomials in all the succeeding phycological literature. They are, partially on this account, much confused. On p. 127 (p. 115 of the reprint) Decaisne also makes it clear that he does *not* consider *Melobesia* (*Mastophora*) *licheniformis*, his type of this genus *Mastophora*, to be synonymous with the *Millepora lichenoides* Ellis et Solander, but that the latter is very similar to his *Melobesia* (sectio I, *Melobesiae verae*) *verrucata* Lamour. Practically every phycologist who has dealt with these matters, from Kuetzing (1849) and Areschoug (1852) to De Toni (1905), has placed the "*Mastophora licheniformis*" Dec'ne under *Lithothamnium lichenoides* (Ell. et Sol.) Heydrich, misled apparently by the similarity of names, and without careful consideration of the complete text of Decaisne.

The genus *Mastophora* Decaisne, fully validated and with its type species, *M. licheniformis* Decaisne, fully illustrated, and with type specimen No. 2232 Cuming from the type locality Manila definitely indicated, has been recognized by all later writers as a proper genus, but the species he referred to it have been very much confused. Their distribution is tropical and subtropical Indo-Pacific. It is impossible to determine with exactness what species are to be recognized and what names are to be applied without a careful study of the types, and this is at present impossible. The following notes, however, may indicate the present complexity of the problem.

The genus *Mastophora* as defined and illustrated by Decaisne seems to be a genus limited to one, or possibly to two, species. It is definitely monostromatic, its layers are not normally superposed, its calcification is strong, so it is not flexible, its branching (or lobing) is lateral and pin-

nate, it is more or less decumbent, and its attachment is through adventitious, elongated unicellular rhizoids with more or less expanded tips. It does not possess a distinct thickened stipe or midribs. Its conceptacles are large, mammiform and strongly projecting, opening in all cases, by a single pore.

Mastophora Decaisne (1842)

Decaisne, *Ann. Sci. Nat., bot.*, ser. 2, 17: 359, 1842; *Melobesia* Sect. III *Mastophora* Decaisne, *Ann. Sci. Nat., bot.*, ser. 2, 18: 126, 1842. *Mastophora* Auctt. recentior., p.p.; *Phlyctidium* Montagne (MS.), *Voy. au Pole Sud.*, 148, 1845.

Mastophora rosea (C. Ag.) comb. nov.

M(astophora) rosea Rosanoff, *Mem. Soc. imp. soc. nat. Cherbourg*, 12: 13, 1866 (?) (nomen!); *Zonaria rosea* C. A. Agardh, *Syst. Alg.*, 264, 1824; Gaudichaud in Freycinet, *Voyage Uranie et le Physicienne, bot.*, 164, 1826. (The names *Padina rosea* Paliser de Beauvois, Ms., and *Dictyota rosea* Lamouroux, *Nouv. Bull. Soc. Philom., Paris*, 1: 33, 1809, while possibly applying to this species or not, were never validated.) C. A. Agardh gives a proper diagnosis. Type locality Guam, in the Marianas (or Ladrones) Archipelago; *Mastophora licheniformis* Decaisne, *Ann. Sci. Nat., bot.*, ser. 2, 17: 359 (p. 63 of reprint) (binomial), 365 (p. 69 of reprint) (genus and type specimen), 380 (p. 84 of reprint), binomial and descr. figures, pl. 17, figs. 11, 11a, 1842 type locality Manila, P. I.; *Melobesia (Mastophora) licheniformis* Decaisne, *Ann. Sci. Nat., bot.*, ser. 2, 18: 126 (p. 114 of reprint), 1842; *Mastophora macrocarpa* Montagne, *Voy. au Pol. Sud*, 149, 1847 type locality Guam, in Marianas (or Ladrones) Arch.; Kuetzing, *Spec. Alg.*, 697, 1849, *Tab. Phyc.*, 8: 48, pl. 100, I, 1858; Arechoug in J. Ag., *Spec. Alg.*, 2: 2: 528, 1852; De Toni, *Syll. Alg.*, 4, 4: 1776, 1905, *Ibid.*, 6, 5: 695, 1924; Heydrich, *Hedwigia*, 33: 300, 1894; Weber von Bosse et Foslie, *Siboga Exp., Mon.* 61: 70, 71, txt. fig. 27, pl. 13, 1904; Setchell, *Univ. Calif. Pub. Bot.*, 12: 109, 1926; *Melobesia foliacea* Kuetz., *Phycol. gen.*, 386, 1843, *Spec. Alg.*, 696, 1849; *Mastophora foliacea* Kuetz., *Tab. Phyc.*, 8: 48, pl. 100, II, 1858 (type locality, Marianas (or Ladrones) Arch., Sonder, *Die Algen von trop. Austral. Abh. Gebiete Naturwissench. Ver. zu Hamburg*, 5 (2): 54, 1871 (locality Cape York); *Mastophora plana* De Toni, *Syll. Alg.*, 4, 4: 1775, 1905 (*non M. plana* Sonder); *Mastophora lichenoides* Auctt. var. p.p.

Mastophora rosea f. condensata (Foslie) comb. nov.

Mastophora macrocarpa forma, Foslie, *Siboga Exp., Mon.* 61: 71, txt. fig. 27, 1904; *Mastophora macrocarpa* f. *condensata* Foslie, *Algol. Notis.*, IV: 30, 1907; De Toni, *Syll. Alg.*, 6, 5: 695, 1924. *Mastophora pygmaea* Heydrich, *Hedwigia*, 33: 300, pl. 15, fig. 16, 1894?

Mastophora tenuis (Decaisne) Aresch.

Areschoug in J. Ag., *Spec. Alg.*, 2, 2: 528, 1852; Kuetzing, *Spec. Alg.*, 697, 1849; De Toni, *Syll. Alg.*, 4, 4: 1777, 1905; *Melobesia* (*Mastophora*) *tenuis* Decaisne, *Ann. Sci. Nat., bot.*, ser. 2, 18: 127 (p. 115 of reprint), 1842, type locality, Hawaiian Islands. This may prove to be a form of the preceding. Specimens from Waikiki, Oahu, Hawaiian Islands, seem referable to *M. rosea*.

Mastophora affinis Foslie

Foslie, *Siboga Exp., Mon.* 61: 71-73, txt. figs. 28, 29, 1904. Type locality, Sikka, Island of Flores, Lesser Sunda Islands. *Mastophora macrocarpa* f. *affinis* Foslie, *Algol. Notis.*, VI: 53, 1909; Foslie (ed. Printz), *Contrib. Mon. Lithoth.*, 47, pl. 74, figs. 7-9, 1929.

This species, together with *M. pacifica* (Heydrich) Foslie from Formosa, seems possibly to be nearer to *Lithoporella* Foslie, but Foslie refers it finally (1909) under *M. macrocarpa*.

Metamastophora gen. nov.

Frons erecta aut adscendens, libera, flexilis, tenuiter calcarea, basi per rhizoides affixa, dichotome aut flabellate ramosa, primo monostromatica mox oligo- usque ad (in stipitibus costisque) polystromatica, cellulis textorum parve differentiatas, in superficiebus ut seriebus successive curvatis apparentibus; rhizoidibus basalibus conceptaculis omnibus magnis, e superficiebus bifariante et impariter prominentibus, poro singulo apertis; tetrasporangiis 4 partitis, conceptaculi fundum totum vestientibus; gonimoblastis periphericis? Genus australe, subtropicum.

Species typica: *Melobesia* (*Mastophora*) *flabellata* Sonder, Australia, S. W.

It seems best to refer the very complex species from South Australia and South Africa placed under the genus *Mastophora* by writers later than Decaisne and which differ fundamentally from the simple *Mastophora rosea*, to a new genus, for which the generic name *Metamastophora* is proposed. Very little study has been made of these species and material of them is infrequent in collections. Much more study is needed of their vegetative structure, of their reproductive organs, and of the development of every kind of conceptacle. Of the probable 5 species, material of only one is accessible. The following outline, therefore, must be regarded as tentative.

A. Branching divaricate, terminal segments broadly cuneate, interruptedly revolute on apex and margins.

1. **Metamastophora flabellata** (Sonder) comb. nov.

Melobesia (*Mastophora*) *flabellata* O. G. Sonder, *Bot. Zeit.*, 1845, 55, type locality, mouth of Swan River, S. W. Australia; O. G. Sonder in Lehmann's *Plantae Preissianae*, fasc. 2: 188, 1847; *Mastophora flabellata* Harvey, *Ner. Austr.*, 108, 1849; Kuetzing, *Spec. Alg.*, 697, 1849, *Tab. Phyc.*, 8: 47, pl. 97, 1858; *Mastophora Lamourouxii* (p.p.), Aresch. in J. Ag., *Spec. Alg.* 2, 2: 526, 1852; Harvey, *Phyc. Austral.*, 5: p. xxx, 1863; De Toni *Syll. Alg.* 4, 4: 1774, 1905, *Ibid.*, 6, 5: 695, 1924; Foslie (ed. Printz), *Contrib. Mon. Lithoth.*, 47 (f. *typica*), pl. 75, figs. 1-3, 1929. S. W. to S. E. Australia.

The Sonder species seems to be an independent entity, but it has commonly been referred under the binomial *Mastophora Lamourouxii*, which will be discussed later on. It may be mentioned here, however, that this binomial is to be considered a *nomen nudum* in the strictest sense. In contrast to the slender South African species referred to Decaisne's second species (*Melobesia tenuis*), the Australian plants of Sonder are relatively firmer, of more than one layer of cells (except at the very tips) and are divaricately flabellate in the lobing of their broadly cuneiform lobes. The plate of Kuetzing (*Tab. Phyc.*, 8, pl. 97, 1858) shows these characters plainly and they are evident in a series of specimens at my disposal in Herb. Univ. Calif. The under surfaces of the segments seem only slightly pruinose.

2. **Metamastophora Lamourouxii** (Dec'ne ex Harvey) comb. nov.

Melobesia (*Mastophora*) *Lamourouxii* Decaisne, *Ann. Sci. Nat., bot.*, ser. 2, 18: 126 (p. 114 of reprint), 1842 (?), nom. nud., *Mastophora Lamourouxii* Endlicher, *Gen. Pl., supp.* 3: 50, 1843 (nomen!); Krauss, *Flora*, 1846, 211 (nomen!); Harvey, *Ner. Austr.*, 108, pl. 41, 1849 (descr. and illustration); Kuetzing, *Spec. Alg.*, 697, 1849 (sub. *Dictyota rosea* Lamour.), *Tab. Phyc.*, 8: 47, pl. 98, II, 1858; Areschoug in J. Ag., *Spec. Alg.*, 2, 2: 526, 1852 (p.p.); De Toni, *Syll. Alg.*, 4, 4: 1774, 1905 (p.p.), *Ibid.*, 6, 5: 695, 1924 (p.p.); Foslie (ed. Printz) *Contrib. Mon. Lithoth.*, 47, excl. plates and references.

The application of the name "*Lamourouxii*" to any *Mastophora* or *Metamastophora* is attended with the utmost of uncertainty. Decaisne does not describe his *Melobesia* (*Mastophora*) *Lamourouxii*, but he does refer to it the *Padina rosea* of Lamouroux in *herb.*, the *Dictyota rosea* Lamouroux, presumably of 1809, but not described, and an indefinite reference to *Peyssonnelia*? of "Dec'ne, Pl. Arab." He definitely separates it from the *Zonaria rosea* C. Ag. (not Lamour.) and seems to indicate that it may occur in the Red Sea, as Kuetzing has suggested. How the name came to be associated with the Port Natal plant, as has been done by Krauss and Harvey, does not appear. Harvey, who seems responsible

for this association, does not indicate as he often does the source of his information. He states only that he has seen dried specimens in the herbarium of Trinity College, Dublin, without indicating whether they were other than those of Gueinzus from Port Natal. One may doubt, therefore, the propriety of quoting Decaisne in connection with this species, unless one has the opportunity of seeing his type specimen.

Whether *Metamastophora Lamourouxii*, of S. E. Africa, obtains the right to its specific name from Decaisne or from Harvey, it certainly appears from the literature to be a distinct species. It seems to differ from *M. flabellata* of Australia by the thinner alae (monostromatic), the lack of divaricate lobing at the extremities of the somewhat less pronounced cuneate blades, and the under surfaces more pruinose. Kuetzing (loc. cit., 1858) certainly represents the alae of this species as monostromatic, while he represents the alae of *M. flabellata* as chiefly di-oligo-stromatic as is indicated by our specimens.

B. Branches oblique, strict; terminal segments not strictly cuneate, entire or lobed at apex, revolute along margins.

3. *Metamastophora canaliculata* (Harv.) comb. nov.

Mastophora canaliculata Harvey in Hook. f., *Flora Tasmanica*, 2: 310, Feb. 15, 1859, *Alg. Austral. Exsicc.*, No. 443, *Phycol. Austral.*, 5, XXXI, pl. 263, 1863; Rosanoff, *Mem. Soc. Imp. Sci. Nat.*, 12: 13, 1866; De Toni, *Syll. Alg.*, 4, 4: 1776, 1905, *Ibid.* 6, 5: 695, 1924; Foslie (ed. Printz), *Contrib. Mon. Lithoth.*, 47, pl. 73, figs. 10-12, 1929.

From the figures of Harvey (1863) and of Foslie (1929), the latter presumably from Harvey's distribution, this species is very distinct in habit and texture, probably approaching the next species in these respects. The dichotomies are obliquely erect, involute or canaliculate throughout, and glabrous and concolorous on both surfaces. Judging from figure 4 of Harvey's plate 263, the alae are polystromatic. This is seemingly a very distinct species, the type specimens from Tasmania, others from neighboring S. E. Australia.

4. *Metamastophora stelligera* (Endlicher et Diesing) comb. nov.

Mastophora stelligera Endlicher et Diesing, *Bot. Zeit.*, 1845, 290 type from Port Natal, South Africa; Kuetzing, *Spec. Alg.*, 697, 1849; Areschoug in J. Ag., *Spec. Alg.*, 2, 2: 528, 1852; De Toni, *Syll. Alg.*, 4, 4: 1777, 1905, *Ibid.*, 6, 5: 695, 1924. *Mastophora hypoleuca* Harvey, *Nereis Austr.*, 108, pl. 41, figs. 1-3, 1849, type locality, Port Natal, South Africa, leg. Dr. Guenzius; Areschoug in J. Ag., *Spec. Alg.*, 2, 2: 527, 1852; De Toni, *Syll. Alg.*, 4, 4: 1775, 1905; Foslie (ed. Printz), *Contrib. Mon. Lithoth.*, 47, pl. 75, fig. 1, 1929.

While it has been impossible to compare the type of the species of Endlicher and Diesing with that of Harvey's species, or, in fact, to examine any specimen at all, it seems proper to consider the two species, identical in type locality and description, as most probably being one and the same. The plants are described as elate, with stipes and branches as narrowly furcate and canaliculate, the terminal blades as lanceolate, with blunt entire or bifid apices, and with margins revolute. The under surfaces of the blades are white-farinose or white-lanate, with depressed dark-colored spots. The conceptacles are described as being uniseriate along both margins of the blades. The upper surface of the blade is dull brownish purple and, according to Harvey, "shagrinated and striate." It seems to be a complex and very striking species if the lower surface is correctly described and the further study of it is most desirable.

C. Branching more or less divaricate; frond flat throughout, and with plane margins; lobes blunt, entire.

5. *Metamastophora plana* (Sonder) comb. nov.

Melobesia (*Mastophora*) *plana* O. G. Sonder, *Bot. Zeit.*, 1845, 55, type locality, mouth of Swan River, S. W. Australia, leg. Preiss.; O. G. Sonder in Lehmann, *Pl. Preiss.*, 2: 188, 1847; *Mastophora plana* Harvey, *Nereis Austr.*, 108, 1849; Kuetzing, *Spec. Alg.*, 697, 1849, *Tab. Phyc.*, 8: 47, pl. 98, I, 1858; Harvey, *Phyc. Austral.*, 5: xxx, 1863; Schmitz und Hauptfleisch in Engler und Prantl, *Die Natürl. Pfl. fam.*, I, 2: 540, fig. 286c, 542, 1897; De Toni, *Syll. Alg.*, 4, 4: 1775, 1905 (p.p.), *Ibid.*, 6, 5: 695, 1924 (p.p.); *Mastophora Lamourouxii* f. *plana* Foslie, *Algol. Notis.*, V: 18, 1908, Foslie (ed. Printz), *Contrib. Mon. Lithoth.*, 47, pl. 75, figs. 4, 5, 1929.

From the descriptions and figures, since no specimens are available, it seems fairly certain that this is a distinct species within the genus. The plane frond is very different from that described for any other species and the figures of Kuetzing, while incomplete as to structure, indicate a high degree of complexity.

The genus *Mastophora*, as founded by Decaisne, in his *Essais*, may be restricted as has been indicated earlier, to species of the simple structure of *M. rosea* (C. Ag.) Setchell. As extended by Endlicher and Diesing (1845), Sonder (1845), Harvey (1849), Kuetzing (1849) and Areschoug (1852), it becomes heterogeneous. Kuetzing, in 1849, included not only the more complex species included here, but also certain species such as: *Mastophora crassiuscula* (Kuetz.) Kuetz. (*Spec. Alg.*, 699, 1849), *M. laevis* (Kuetz.) Kuetz. (*Ibid.*, 699; *Lithophyllum laeve* Kuetz., *Bot. Zeit.*, 5: 33, 1847, non *Lithophyllum laeve* Stroenfelt, *Algueg. Isl.*, 21, pl. 1, figs. 11, 12, 1886, a later homonym); *M. lichenoides* (Ell. et Sol.) Kuetz. (not

M. licheniformis Dec'ne), and *M. patena* (Harv.) Kuetz., as a first group and, as a second group, the several species later described by Foslie and referred to *Lithoporella* and *Litholepis*. The members of the second group are to be considered properly referred as will be shown later. The members of the first group are mostly of a definite life form and hemiparasitic. In another paper they will be referred to a new genus. Very puzzling is the *Mastophora* (*Lithostrata*) *lapidea* Foslie (*Algol. Notis.*, II: 27, 1906) which was later referred to *Lithoporella* (*Lithostrata*) *lapidea* Foslie (*System. Bemerk.*, in *Det. Kongl. Norske Vidensk. Selskabs Skrifter*, 1909 (2): 59, 1909) and finally as "*Mastocarpus lapidea* Harv." Foslie (in *Contrib. Mon. Lithoth.* (ed. Printz), p. 47, pl. 73, figs. 5-7, 1929). No description of the fruiting organs is given nor any reference to the genus *Lithostrata* Heydrich (1905) of the *Squamariaceae*. The only suggestion that may be made is that, without examination of the type, it possibly may represent a species of Foslie's first proposal (1898) of *Goniolithon* (subgen. *Eugoniolithon*) and that it may be related to *G. papillosum* (Zan.) Foslie.

In "1902" (or more probably in 1903) Foslie (in *Aarsberetning for "1902" in Det. Kongl. Norske Vidensk. Selskabs Skrifter*) outlined some 8 groups, into which he proposed to divide the *Corallinaceae*. In that outline he proposed the "Mastophoreae" to include the genus *Mastophora* (in an extended sense). He gave no characters for his groups but only enumerated the genera to be included under each. He named special groups for each of the definitely parasitic genera: *Schmitziella*, *Chaetolithon* and *Choreonema*. He also included, but doubtfully, "*Hildenbrandia*."

The other 3 groups proposed by Foslie are *Lithothamnioneae*, *Melobesieae* and *Corallineae*. Disregarding the parasitic (or hemiparasitic) nature of the genera as group characteristics, and reviving some names in accordance with the "Rules of Nomenclature," there may be proposed a series of 5 groups, or subfamilies, as follows:

1. Frond crustiform to fruticulose, inarticulate, continuous (reduced in parasitic species).....2
1. Thallus articulate or jointed.....5. *Corallineae*
 2. Thallus monostromatic to partially polystromatic, but without differentiation of tissues.....4. *Mastophoreae*
 2. Thallus di-polystromatic, with differentiated tissues.....3
3. Tetrasporangia in sori.....1. *Sporolithaeae*
3. Tetrasporangia in conceptacles.....4
 4. Tetrasporangial conceptacles opening by few to many pores.....
 -2. *Lithothamniaeae*
 4. Tetrasporangial conceptacles opening by a single pore.....3. *Lithophylleae*

The *Mastophoreae*, differing from all other *Corallinaceae* by their lack of, or, at least, by their very feeble differentiation of tissues, include 5 genera, arranged as follows:

1. Fronds decumbent or ascending, monostromatic, brittle.....2
1. Fronds elate, becoming homogeneous—polystromatic, with stipe branching and terminal blades flexible.....5. *Melamastophora*
2. Fronds entire on margins, proliferating obliquely and lamellately, producing superposed layers devoid of rhizoids.....3
2. Fronds lobed on margins, neither proliferating nor superposed, attached by adventitious rhizoids.....4. *Mastophora*
3. Fronds superposed, agglutinated into a common crust.....3. *Goniolithon*
3. Fronds superposed, free.....4
4. Thallus cells palisade-like.....2. *Lithoporella*
4. Thallus cells nearly isodiametric.....1. *Litholepis*

THE PEDIGREED CULTURE OF *PARAMECIUM AURELIA* AT YALE UNIVERSITY

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It seems desirable, as a matter of record, to bring up to date the history of the long-continued pedigreed culture of *Paramecium* at Yale University, because it is now more than two decades since a summary appeared in these PROCEEDINGS.¹

The culture was started on May 1, 1907, by the isolation of a "wild" specimen of *Paramecium aurelia* on a depression slide in about five drops of culture fluid. Descendants of this individual constitute the various lines of the pedigreed race. During the first eight months the culture medium consisted solely of hay infusion, but thereafter infusions of various materials common in the usual environment of *Paramecia* proved to be more favorable and were employed. The media have always been thoroughly boiled to prevent any possible contamination with foreign strains of *Paramecium*. In brief, the cells of the culture today are direct lineal descendants by division from the single animal isolated in 1907.

The main object of starting the culture was to determine whether *Paramecium* can reproduce by division indefinitely without recourse to conjugation. Therefore during the first eight years of the culture's life, to May 1, 1915, during which 5071 generations were attained, the possibility of conjugation was absolutely precluded by the daily observation and isolation of the products of division. After this date daily isolation was judged unnecessary, because the continued life and health of the culture had long since justified the conclusion that conjugation is not, as had been previously generally maintained, a *sine qua non*, under favorable environmen-

tal conditions, for the continued life of *Paramecium* and, presumably, of unicellular animals in general.

However, at the formal termination of the experiment in 1915, the culture was still maintained but without the exacting daily observation and recording of the division rate previously required. So, from this point, there is the possibility that conjugation between closely related individuals of the pedigreed culture may have occurred without being detected.

In this manner the culture has been maintained up to the present (March 1943). From time to time, thirty-day tests have been made of the division rate of the animals under the former rigid culture conditions, and in nearly every case the same general average division rate has been revealed as during the first eight years of life; that is, between 50 and 60 generations per month. There is no evidence of waning vitality. On the basis of these tests it is fair to estimate 600 generations attained each year since May 1, 1915, which gives, in round numbers, 21,800 generations attained by the culture during the nearly 36 years of its life to date. The vitality of the culture is further attested by the fact that it is continuously affording animals for various other experiments in the Osborn Zoölogical Laboratory and elsewhere.

It may be recalled that studies on this culture by Woodruff and Erdmann² revealed a periodic internal nuclear reorganization process to which the name endomixis was given. The establishment of endomixis raised new problems though obviously without affecting the basic conclusion that conjugation is not intrinsically a necessary phenomenon in the life history of the organism. If, however, it prove true that synkaryon formation may occur during reorganization in this race, as first stated by Diller,³ so that the process becomes autogamic, then, of course, *self-fertilization* has not been excluded in the experiments.⁴

¹ Woodruff, L. L., "The Present Status of the Long-Continued Pedigreed Culture of *Paramecium aurelia* at Yale University," *Proc. Nat. Acad. Sci.*, **7**, 41-44 (1921).

² Woodruff, L. L., and Erdmann, R., "A Normal Periodic Reorganization Process without Cell Fusion in *Paramecium*," *Jour. Exp. Zool.*, **17**, 425-518 (1914).

³ Diller, W. F., "Nuclear Reorganization Processes in *Paramecium aurelia*, with Descriptions of Autogamy and Hemixis," *Jour. Morph.*, **59**, 11-67 (1936).

⁴ Woodruff, L. L., "Endomixis," *Protozoa in Biological Research*, edited by G. N. Calkins and F. M. Summers, Columbia University Press, 1941, pp. 646-665.

HIGH MUTATION FREQUENCY IN DROSOPHILA PSEUDOÖBSCURA, RACE B

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Variations in the mutation rate may be due to a number of factors in the external or internal environment of the organism. We can control many of these factors; others are unknown and appear spontaneously; they may persist for some time and then disappear again. In some cases this may be due to a selection of proper genic modifiers of the mutation rate. Modifying genes have been made responsible for the control of the mutation rate, since the mutation rate can be altered by hybridization; such a case has been described for *Drosophila pseudoöbscura*.¹ Several investigators have observed cases of high mutation frequencies in *D. melanogaster*^{2, 3, 4}; other cases of the same nature are more doubtful. *D. pseudoöbscura*, race B, has furnished another example exhibiting the sudden rise of mutation frequency, to be recorded in this paper.

The phenomenon first appeared in the progeny of a pair mating, where four sex-linked mutants were observed. On outcrossing flies from this culture, mutations occurred again to an unexpected degree. The number of mutations per pair mating is quite variable. Some of the most striking cultures are recorded in table 1. In each case we deal with the progeny of only one pair mating. The average yield per culture is 242 flies; this number is based on counts of the progenies of 758 pair matings chosen at random; even in the largest progenies there are less than 500 flies per bottle.

TABLE 1

CULTURE NO.	TYPE OF MUTATION	NO. OF MUTATIONS
Original	<i>bd, y, ri</i> , (spread wings)	4
1045	<i>v, Aw, L</i> , (Abnormal)	4
1176	<i>bd^{cl}, dx, dy(m), Sc</i>	4
1671	<i>dy(m), L, Vg</i> , (Abnormal)	4
1691	<i>bd, scl, tt, Cy</i> , (Wing-mosaic)	5
1697	<i>bd, scl, com, asc, Sc</i> , (rough eye)	6
2654	<i>bd, dy(m), bbl, Aw</i> , (rough eye)	5
2676	<i>scl, w, sn(f), Aw, tt</i>	5
2708	<i>dy(m), tt, Tho</i> , (Rough eye)	4
2936	<i>bd, scl, Vg</i> , (Rough eye)	4
2971	<i>bd, com, H^A, Sb, Vg</i>	5
a177	<i>dy(m), com, asc, ju, dow</i> , (rough eye)	6

The mutations evidently may occur at any time during the development of the germ line or of the somatic line. Mutated individuals of a certain type may occur in numbers varying from one to fifty per cent of the offspring of a pair mating; in somatic mutations patches of varying sizes have been observed. There seems to be no preference as to the time of mutation. The numbers of mutations recorded in this paper refer to separate occurrences of mutation, not to numbers of individuals showing new mutant characters.

In our study only "visible" sex-linked recessives are used as indicators of the high mutation rate, because they are detected in the immediate offspring of a female with the abnormal rate. Autosomal recessives should not be used, because the probability of their detection depends on the mating system used, and even then it is not certain at what time and in which individual they have arisen. Dominants are disregarded to avoid confusion with another mechanism which is responsible for inducing mutations of the deficiency type; we shall refer to this later. Although the high mutation rate is observed in males also, and although dominants and autosomal recessives are produced by the present mechanism, it is for the above reasons that we restrict our observations to visible sex-linked recessives arising in females.

The most likely cause of a phenomenon of this nature is a genic one. It has proved difficult, however, to demonstrate that a gene is responsible for the increase in the mutation rate. We deal here with a quantitative difference between the normal and the mutating strains; therefore, large numbers are required to make the data meaningful.

The normal spontaneous mutation rate for visible sex-linked recessives in *D. pseudoobscura*, race B, is 0.01%. This number is based on the occurrence of four sex-linked recessives in 46,804 flies, from cultures preceding the time of the first observation of the high mutation rate, plus those from the pure Seattle strain.

In determining the abnormal mutation rate we shall consider only those cultures which have given at least one sex-linked recessive, since this is the only indication of the presence of the "Mutator" gene in the mother. Thus, we obtain a mutation rate per culture showing mutations, rather than a mutation rate per total number of flies. The higher the total mutation rate, the greater will be the coincidence of several sex-linked recessives in one culture, remembering that we always deal with the offspring of one female per culture and that the average number of offspring is the same for wild type females and Mutator females. With a normal mutation rate of 0.01% for sex-linked recessives it is obvious that two mutations would occur by chance in the same culture so rarely that the mutation rate per culture showing mutations is practically 1.00. Thus, from Seattle females seven mutations were obtained in seven cultures (rate = 1.00); from Morro fe-

males five mutations were obtained in five cultures (rate = 1.00). But from selected females 160 mutations were obtained in 102 cultures (rate = 1.57). The coincidence of mutations is directly related to the total mutation rate, so that by knowing one we can determine the other. Due to the small numbers, which are always a disadvantage in a study of this sort, the errors may be considerable; the calculations are also based on the assumption of a constant number of flies per bottle, which further decreases the accuracy of the result. Therefore, the rates which will be presented must be taken as approximations; but we are interested in getting some idea of the mutation frequency in flies heterozygous or homozygous for the Mutator gene. In general, the estimates are probably conservative, since the normal spontaneous mutation rate is included in the abnormal rate, which would tend to lower the coincidence.

Females resulting from a mating of a selected fly to wild type, regardless of the direction in which the cross is made, give a rate of 1.47 (100 mutations in 68 bottles giving mutations). This is the rate obtained from flies heterozygous for the Mutator gene. Assuming a Poisson distribution, that is, a population homogeneous for a factor increasing the mutation rate, a result like this would be obtained, if the rate of mutations per total number of flies were 0.34%. Thus, the normal spontaneous mutation process is increased thirty-four times with one dose of the Mutator gene.

If flies can be homozygous for the Mutator gene, we might expect a higher mutation rate from females that have resulted from a mating of both selected male and female; or, conversely, only if flies resulting from such a mating give more mutations, can we know that homozygous flies are produced and that they have a higher mutation rate than the heterozygous flies. Females resulting from such a mating give a rate of 1.76 (60 mutations in 34 cultures giving mutations). That is the rate obtained from females both of whose parents were probably heterozygous for the Mutator gene. From that mating we should expect one-fourth of the offspring to be homozygous for the Mutator gene one-half heterozygous and one-fourth wild type.

Let m be the expected mean frequency of occurrence of a mutation in the population of subsamples. Since m is known to be equal to 0.0034×242 for cultures resulting from heterozygous females, and equal to 0.0001×242 for cultures resulting from homozygous wild type females, the relative frequencies of subsamples with one mutation, two mutations, etc., are known for these two groups. Let k be the number of cultures resulting from homozygous Mutator females; then there are $k \frac{m}{e^m}$ cultures with one mutation in this group, $2k(0.3615)$ cultures with one mutation when the mother was heterozygous and $k(0.0234)$ cultures with one mutation when the mother was wild type; similarly for cultures with two, three, etc.,

mutations. We equate the theoretical total of cultures with one mutation to the observed number with one mutation, and do likewise for the cultures with two, three, etc., mutations; we obtain the following six equations:

$$k \frac{m}{e^m} + 2k(0.3615) + k(0.0234) = 21$$

$$k \left(\frac{m^2}{2e^m} + 0.2979 \right) = 7$$

$$k \left(\frac{m^3}{6e^m} + 0.0960 \right) = 2$$

$$k \left(\frac{m^4}{24e^m} + 0.0168 \right) = 2$$

$$k \left(\frac{m^5}{120e^m} + 0.0028 \right) = 1$$

$$k \left(\frac{m^6}{720e^m} + 0.0004 \right) = 1$$

The number of mutations in the homozygous group is equal to km , in the heterozygous group it is equal to $2k(242)$ (0.0034), and in the wild type group it is equal to $k(242)$ (0.0001); the total number of mutations obtained was 60; therefore, we find $km + 2k(242)$ (0.0034) + $k(242)$ (0.0001) = 60, or, $k = \frac{60}{1.67 + m}$. We can now determine the most probable value

of m in the usual manner.⁶ We find $m = 1.703$. Thus, the mutation rate of females homozygous for the Mutator is 0.70%, approximately twice that of the heterozygotes. This is a linear increase with the dosage. In this connection, it is interesting that the gene dotted in maize produces an exponential increase with the dosage as concerns mutations of the "a" gene.⁶

The offspring of heterozygous flies should have a total mutation rate of 0.17% if the above calculations are correct, since now half the flies are heterozygous and half are wild type; therefore the mutation rate should be one half of 0.34%. We actually obtain 7 mutations in 4679 flies which is a rate of 0.15%; this is in good agreement with the expectation.

The crosses designed to locate the Mutator gene are shown in table 2. All the females shown in the table should be heterozygous for the Mutator gene except those with the wild type chromosome whose homologue is responsible for carrying the Mutator gene; from these females we should expect a rate of 1.00 whereas all the others should give an average of 1.47. Thus, chromosome II would seem to be the carrier of the Mutator gene.

This, however, must be taken cum grano salis; not only are the numbers uncomfortably small for this kind of a test, but the dot chromosome which cannot be marked was not controlled. The conclusion, therefore, is tentative.

TABLE 2

Selected Stubble (II) or Scute (III) or Curly (IV) × Morro or Seattle			
<div> <div>Stubble or Scute or Curly</div> <div>Morro or Seattle</div> </div> <div>♂ ♂ × Morro or Seattle ♀ ♀</div>			
	NO. OF MUTATIONS	CULTURES SHOW- ING MUTATIONS	RATE
Selected II	23	14	1.64
Wild type II	4	4	1.00
Selected III	12	9	1.33
Wild type III	6	4	1.50
Selected IV	15	8	1.86
Wild Type IV	15	11	1.36

Table 3 shows the maps of the chromosomes; the map of the *X*-chromosome is based partially on the one published previously.⁷ Not all of the mutants shown on the maps can be assumed to have been induced by the Mutator gene here under discussion; there is another mechanism at work in the same cultures which induces an extraordinarily high number of mutations of the deficiency type, mainly Smoky, Notch and Minutes. Sometimes it is difficult to tell the two apart, because in some cases they have nearly the same effect. Description of the other mechanism will have to wait until a later date; it is believed that the sex-linked and autosomal recessives and many of the dominants have been induced by the Mutator gene here under consideration.

Since tests for allelism were not generally applied, the number of occurrences of a certain mutation can be given only for those loci where identification by phenotype is not likely to lead to confusion with other mutants. The asterisks indicate that the mutation has occurred at least once, but the nature of the mutant does not permit identification by inspection. Thus, dusky and miniature cannot be distinguished phenotypically; although many mutations of this type have appeared, it cannot be stated how many of each. The bar signifies that the mutant has not been observed.

The mutants were given the names of the *D. melanogaster* mutants which they resemble most closely. Some cannot readily be compared. Hairless^A is a dominant, lethal to the male. Both prune alleles always show a distinct mottling effect. The deltex alleles are female sterile and semilethal

TABLE 3

X-CHROMOSOME			II CHROMOSOME		
lozenge (<i>lz</i>)	0.0	*	Smoky (<i>Sm</i>)	0.0	40
almondex (<i>amx</i>)	3.6	*	Thorax (<i>Tho</i>)	5.1	2
beaded (<i>bd</i>)	31.1	50	Stubble (<i>Sb</i>)	6.1	10
Minute ⁴ (<i>M</i> ⁴)	35.5	*	Hairless ^E (<i>H</i> ^E)	14.9	*
Hairless ^A (<i>H</i> ^A)	54.1	*	bithorax (<i>bx</i>)	39.2	—
scutellar (<i>scl</i>)	61.9	39	glass (<i>gl</i>)	42.1	*
yellow (<i>y</i>)	61.9	6	cinnabar (<i>cn</i>)	54.7	—
prune (<i>pn</i>)		2			
deltex (<i>dx</i>)	64.7	3	Minute ⁸ (<i>M</i> ⁸)		*
Notch (<i>N</i>)		24	Minute ¹⁴ (<i>M</i> ¹⁴)		*
white (<i>w</i>)	69.6	3	Mutator (<i>Mu</i>)	?	
<i>lm₁B</i>		—			
			III CHROMOSOME		
singed (<i>sn</i>)	71.0	*			
vermillion (<i>v</i>)	72.8	*			
Filiform (<i>Ff</i>)	72.9	*			
lethal plexus (<i>lp</i>)	73.1		Abbreviated (<i>Abb</i>)	0.0	*
Fused ² (<i>Fu</i> ²)	73.5	*	Lobe (<i>L</i>)	29.2	*
dusky (<i>dy</i>)	73.6	*	Scute (<i>Sc</i>)	50.5	*
miniature (<i>m</i>)	73.6	*	Vestigial (<i>Vg</i>)	50.5	*
forked (<i>f</i>)	74.1	*			
rose (<i>rs</i>)	77.2	—	gap (<i>gp</i>)		*
tilt (<i>tl</i>)	83.7	11	orange (<i>or</i>)		*
bubble (<i>bbl</i>)	91.6	*	Minute ⁵ (<i>M</i> ⁵)		*
fused ¹ (<i>fu</i> ¹)	92.3	*	Minute ⁶ (<i>M</i> ⁶)		*
compressed (<i>com</i>)		5	Minute ⁷ (<i>M</i> ⁷)		*
ascute (<i>asc</i>)	98.9	10	Minute ¹⁰ (<i>M</i> ¹⁰)		*
Abnormal wing (<i>Aw</i>)	99.8	7	Minute ¹² (<i>M</i> ¹²)		*
Curvoid (<i>Cur</i>)	106.2	*	Minute ¹⁵ (<i>M</i> ¹⁵)		*
scarlet (<i>st</i>)	119.3	*			
<i>lm₂B</i>		—			
			IV CHROMOSOME		
sepia (<i>se</i>)	149.0	—			
radius rudimentary (<i>rr</i>)	171.4	—			
javelin (<i>ju</i>)	176.2	*	Minute ⁴ (<i>M</i> ⁴)	0.0	*
short (<i>s</i>)	181.8	3	Scutellum diminished (<i>Sd</i>)	32.9	*
			Hairless ^B (<i>H</i> ^B)	57.2	*
radius incompletus (<i>ri</i>)		*	Curly (<i>Cy</i>)	69.7	*
shiny (<i>shi</i>)		*	Dachsoid (<i>Dsd</i>)		*
tiny bristle (<i>tb</i>)		*			
condensed (<i>con</i>)		*	net (<i>net</i>)		*
roughex (<i>ru_x</i>)		*	dachs (<i>d</i>)		*
notchy (<i>ny</i>)		*	Large wing cells (<i>Lwc</i>)		*
downy (<i>dow</i>)		*	Enhancer-glass (<i>E-gl</i>)		*
diminutive (<i>dm</i>)		*	Minute ¹ (<i>M</i> ¹)		*
small bristle (<i>sbr</i>)		*	Minute ² (<i>M</i> ²)		*
ruby (<i>rb</i>)		*	Minute ⁹ (<i>M</i> ⁹)		*
approximated (<i>app</i>)		*	Minute ¹¹ (<i>M</i> ¹¹)		*
twisted (<i>tw</i>)		*	Minute ¹³ (<i>M</i> ¹³)		*

to the male. Abnormal wing has large wing cells giving the wing a coarse appearance; delta or net type of venation is common; it is usually lethal to the male. In shiny the chitin has a polished appearance. Thorax shows a longitudinal median groove of the thorax as the most striking characteristic. Hairless^B rarely survives when homozygous; in the homozygous state it shows extreme aristopedia effects; the heterozygous flies sometimes have the aristae reduced or absent; the legs also are sometimes reduced. Vestigial takes off some bristles along the costa when heterozygous. Scutellum diminished affects the size of the scutellum which may be practically absent in extreme cases. Hairless^B resembles heterozygous Hairless^E. Dachsoid shortens the legs and roughens the eyes. Large wing cells resembles Abnormal wing in its phenotypic effect. Enhancer-glass allows heterozygous glass to express a rough eye.

In addition to the mutants shown in the maps there has occurred a large number of mutants which were either inviable or sterile, lost or discarded before being mapped, or which have not yet been mapped. The total number of observed mutations is approximately two thousand.

Aside from the evolutionary significance of Mutator genes it is interesting to speculate about their mode of action. From the nature of the mutations which are induced by this mechanism it seems obvious that we are dealing with true point mutations. Mutator genes probably act through a chemical medium. Chemicals are the least satisfactory agents of the more important ones which have been investigated as to their faculty of inducing mutations. It is possible that Mutator genes interfere with the proper reproduction of genes and that by learning the nature of this mechanism we may know more about the chemistry of gene reproduction. Mutator genes rather than producing certain substances might be responsible for the lack of a substance necessary for growth and reproduction of genes in general. There is no good chance at this time to learn much about Mutator genes except the result of their activity, unless the substance involved were in the nature of a hormone; this is unlikely, since intranuclear processes are concerned. It must be left to future research further to illuminate this problem.

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Summary.—1. *Drosophila pseudoöbscura*, race B, has furnished certain stocks with an abnormally high mutation rate.

2. The mutations may occur at any time during the development of the germ line or of the somatic line.

3. The phenomenon is due to a dominant "Mutator" gene which increases the normal spontaneous rate about thirty-four times when heterozygous, and about seventy times when homozygous; this represents a

linear increase in the mutation rate with the dosage of the Mutator gene.

4. The Mutator probably is linked to the second chromosome.

5. A total of approximately two thousand mutations was observed; some of the mutations were located.

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ON THE PHYSICAL BASIS FOR GENETIC RESISTANCE TO MOUSE TYPHOID, *SALMONELLA TYPHIMURIUM**

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With most species wide variations in susceptibility to most diseases exist. Some members of the species die, others show different degrees of morbidity. By suitable genetic techniques it is possible to segregate these different levels of disease resistance into consistently breeding groups, some with nearly complete mortality, some of medium resistance, others with little or no morbidity when exposed to the same dose of the causative organism. Using the mouse, *Mus musculus*, as the host and the typhoid organism, *Salmonella typhimurium*, as the disease causing organism investigations at Iowa State College^{1, 2} have segregated a mouse population into six different breeding groups characterized in part by their disease resistance. The first two groups designated as *Ba* and *L* have a mortality of 92 and 87 per cent, respectively, when inoculated with 200,000 bacteria; the *E* and *Z* lines have 47 and 42 per cent mortality; and the *R.I.* and *S* lines have 25 and 14 per cent. Each line is now essentially pure breeding for its resistance to *Salmonella typhimurium*. The purpose of this investigation was to investigate one of the several possible physiological causes for this resistance and thus establish a character basis for the observed genetic resistance. The character chosen for study of this correlation is the cellular constitution of the mouse blood.

For the purposes of this analysis the blood of 45 to 50 mice of each strain was examined for the number of erythrocytes, leucocytes and their proportions.

In our mice the cellular constituents of the blood are shown to vary widely, both in total number and the proportion of the different cell types. This variation seems to be characteristic of blood in general, as we have found similar variations in the published results of other investigators. Two major variables and one minor are known contributors to the variation. The major variables are sex of the mouse and the strain from which it is derived. The minor variable is the short age range over which the mice were tested. Analysis of the effect of these variables proves that sex is relatively unimportant in its influence on either the numbers of erythrocytes or leucocytes or the proportion of the cells composing the leucocytes. In its narrow range, age also is not particularly important as the variation attributable to age is irregular, not showing a consistent trend. Genetic differences which have been segregated into the different strains composing our material account for most of the known variation in the numbers of erythrocytes and leucocytes. The same genetic differences affect the proportions of the cell types composing the leucocytes to only a limited degree.

If we contrast the six different strains we find that the levels of the erythrocytes and leucocytes in the blood are characteristic of the particular strains. In the breeding process the variations of these cells in different mice have been reduced and made characteristically higher or lower in the particular strain. It is found that there is no correlation between the degree of fixation of the erythrocytes and the leucocytes indicating that for the six strains they have been fixed independently of each other. The proportions of the different cells composing the leucocytes do not show the same degree of fixation in this hereditary process as do the cell numbers.

The variation in numbers of leucocytes is highly correlated with the degree of resistance which exists for the particular mouse strain (Fig. 1). Those strains of low resistance have low leucocyte numbers whereas those of high resistance have relatively high leucocyte numbers within the range of the customary variation. In the formation of these strains of mice, those of high and those of low resistance have been established through inbreeding and selection for either high survival value or low survival value to the typhoid disease organism. In the process the leucocyte numbers have also been fixed, the correlation between the leucocyte number for the given strain and its resistance being on the order of 0.9. This fact would be as expected if, basically, there was a direct relation between the number of leucocytes which the organism carried and its potentialities for resisting mouse typhoid.

At the same time that these breeding experiments for disease resistance were going on, inbreeding experiments having no particular relation to disease resistance were also molding other different mouse strains. This inbreeding technique should also lead to fixation of particular types of re-

sistance or leucocyte numbers. Such is found to be the case. One of these strains having a low leucocyte number has high susceptibility to mouse

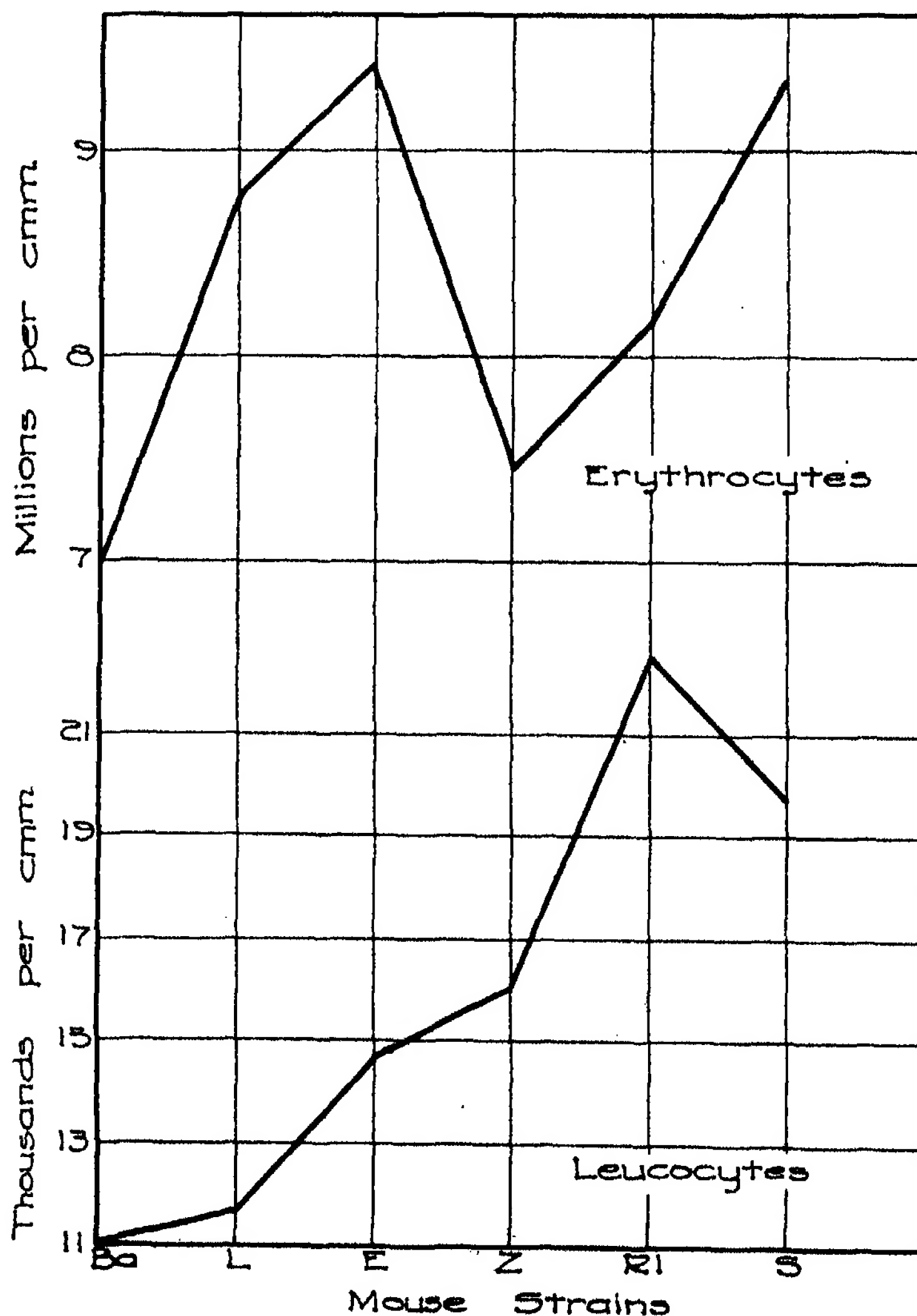


FIGURE 1

Relation of the disease resistance of the different mouse strains to erythrocyte or leucocyte number found in their blood.

typhoid, whereas two of the other strains have intermediate leucocyte number and intermediate susceptibility.

The proportion of the particular types of cells which make up the leucocytes are not fixed in the breeding process. This suggests that the particular cell types are called out by the animal body through different environmental conditions and that a primary cell is capable of developing into any particular type according to these environmental influences rather than through the inheritance control. Such a view would indicate that the important inherited capacity is the production of a few or large number of primary cells that subsequently may develop into leucocytes of the various types according to the environmental need.

The erythrocytes show a similar fixation in their numbers to that of the leucocytes. They do not, however, show any particular correlation to the disease resistance of the given strain. This we might possibly expect in view of the fact that the erythrocytes, so far as mouse blood is concerned, are not known to effect directly the typhoid organism.

In this search for the physical basis of the genetic resistance to mouse typhoid we have studied only two possible types of cells out of many which the body could furnish and be important to the resistance. Both cell types studied have a large variability indicating that there are both hereditary and environmental causes of variation. Analysis shows that our genetic technique would account for about one-fourth of the variations normally present in these mouse blood cells. The other three-fourths of the variation must be due to causes other than those measured in these experiments. The proportions of the different types of cells making up the leucocytes are but little fixed by the genetic techniques used in establishing the different strains.

Our observations have interesting corollaries in the work of Reich and Dunning³ on the effect of leucocyte level and longevity in rats. Six closely inbred lines of rats were tested for the numbers of white blood cells, the per cent of polymorphs and the duration of life. It was found that the higher the leucocytes the longer the rat lived. It was also shown that the neutrophile polymorph represented a higher proportion of the leucocytes in the longer lived rats than in those with a shorter duration. The correlation is of the order of 0.7. Contrary to our findings a sex effect was noted in the leucocyte count and the duration of life. These sex effects favor larger numbers of leucocytes in the females, the sex with the longer duration of life.

Roberts, Severens and Card⁴ present a study of the nature of the hereditary factors for resistance and susceptibility to pullorum in the domestic fowl. In this study they analyze the numbers of erythrocytes, leucocytes, lymphocytes and neutrophiles from the fifteenth day of incubation to a week after the chick hatches. It is in this period that the chicks of susceptible strains are killed by *Salmonella pullorum*. After this period most strains of chickens, both susceptible and resistant, are immune to this dis-

ease. The total number of leucocytes is found to increase in both susceptible and resistant strains from the fifteenth day of incubation through the seventh day after hatching. This increase is accounted for in part by a shift in the types of cells composing the leucocytes. The lymphocytes increase from 5 or 10 per cent up to 55 to 65 per cent of the total leucocytes. The chicks which are genetically resistant to pullorum display this rise in lymphocytes earlier than the chicks which are susceptible. The resistant and susceptible chicks have essentially the same lymphocyte numbers seven days after hatching, the period after which both groups are resistant to this disease.

The importance of the lymphocytes is further brought out by x-ray experiments in which the lymphocytes were reduced in number through irradiating the 6-day-old chicks with x-rays. These x-ray chicks, inoculated with pullorum, had a death rate four times that of the untreated birds. These results would point to the leucocytes as important in the defense mechanism to this disease, especially as no difference in bactericidal power of the serum of the susceptible and resistant groups was observed.

In their study of the correlation between the resistance to rat typhoid and bactericidal power of whole blood, Irwin and Hughes⁶ showed that rats which were resistant to the disease had less bacteria in their sodium citrated blood, after inoculation with *Salmonella enteritidis* and incubation at 38° for four hours than rats which were incapable of surviving the disease. As the paper stands it is not possible to decide whether this *in vitro* action is due to the serum or to the presence of leucocytes. We are informed, however, that the serum is probably the responsible agent here.

Rous and Jones⁶ have presented a study of the *in vitro* reactions of leucocyte, bacteria or other antigens and immune serum. In this paper they properly emphasize the fact that after one hour's incubation bacteria or red blood cells ingested by leucocytes are protected against the action of immune serum in causing bacteriolysis or hemolysis. They point out the significance of this fact to possible bacterial dissemination within the host should the pathogen be eventually freed from the leucocytes. The protective action against immune sera by the leucocytes is found to be a property of the living organism not of the dead leucocytes. The hypothetical significance of these researches to the possible *in vitro* reactions of the leucocytes to disease organisms is of marked significance to our results. The experimental arrangement is excellent. The experiments present data on pathogen death, or erythrocyte hemolysis, when leucocytes are present in the mixture contrasted with their absence from the mixture. In this light the data show that the leucocytes destroy or immobilize many of the bacteria. This reaction may, of course, be looked upon as an exaggerated form of agglutination since each leucocyte collects a fairly large number of bacteria within it. However, the microscopic examination of ingested

bacteria and erythrocytes would indicate rather that the leucocytes' cytoplasm destroys the inclusions rather than simply agglutinating them.

The general evidence of the foregoing papers indicates that the numbers of leucocytes in general, or numbers of particular kinds of leucocytes, play a pronounced part in the immune phenomena controlled by the genetic constitution of the host.

The original data with its complete analysis will appear shortly.

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BIO-ASSAY ON A GENERAL CURVE

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In the discussion of bio-assay we have used the growth curve¹ where others have used the probability integral. Some of the treatment may be given in terms of a general function F , and for the study of the differences which might be introduced by different laws of biological reaction of various animals to various biologicals such a discussion is not without importance. Let it, then, be supposed that

$$P = 1/2 + 1/2 F[\alpha(x - \gamma)], \quad Q = 1/2 - 1/2 F[\alpha(x - \gamma)]$$

in which α is a coefficient of homogeneity and γ is the 50% point for x so that $F(0) = 0$. For the growth curve and the probability curve we have, respectively,

$$F(z) = \tanh z \quad \text{and} \quad F(z) = \frac{2}{\sqrt{2\pi}} \int_0^z e^{-z^2/2} dz.$$

For each of these curves there is no limit to the values of z , but for some biologicals there might be a minimum dose necessary to produce any effect and a maximum dose which would affect all animals, and in that case z would effectively range only between finite limits. Three hypothetical cases might be

$$F(z) = z, \quad F(z) = \sin z \quad \text{and} \quad F(z) = \frac{z}{\sqrt{1+z^2}}$$

in the first of which z would range from -1 to $+1$, in the second from $-\pi/2$ to $\pi/2$, and in the third again from $-\infty$ to $+\infty$ but with the order of contact of $F(z)$ with its asymptotes much lower than for the growth curve.

The likelihood is

$$L = \sum s_i \log(1 + F_i) + \sum (n - s_i) \log(1 - F_i).$$

The equations which determine the parameters α , γ are

$$\frac{\partial L}{\partial \gamma} = -\alpha \sum \frac{2s_i F_i'}{1 - F_i^2} + n\alpha \sum \frac{F_i'}{1 - F_i} = 0, \quad (1)$$

$$\frac{\partial L}{\partial \alpha} = \sum \frac{2s_i (x_i - \gamma) F_i'}{1 - F_i^2} - n \sum \frac{(x_i - \gamma) F_i'}{1 - F_i} = 0. \quad (2)$$

In general even for the case of three dilutions and $x_i = x - c, x, x + c$, these equations are not generally solvable when F has a specified form.²

Differentiating again, we have

$$\frac{\partial^2 L}{\partial \gamma^2} = \alpha^2 \sum \left[\frac{2s_i F_i''}{1 - F_i^2} - \frac{n F_i''}{1 - F_i} + \frac{4s_i F_i'^2 F_i}{(1 - F_i^2)^2} - \frac{n F_i'^2}{(1 - F_i)^2} \right].$$

If we substitute for s_i the value $n[1/2 + 1/2 F_i]$ which it has on the fitted curve, from which any particular set of values s_i is considered to have arisen through sampling errors,

$$\frac{\partial^2 L}{\partial \gamma^2} = -n\alpha^2 \sum \frac{F_i'^2}{1 - F_i^2} \quad (3)$$

and in a similar manner

$$\frac{\partial^2 L}{\partial \gamma \partial \alpha} = n\alpha \sum \frac{(x_i - \gamma) F_i'^2}{1 - F_i^2}, \quad \frac{\partial^2 L}{\partial \alpha^2} = -n \sum \frac{(x_i - \gamma)^2 F_i'^2}{1 - F_i^2}. \quad (4)$$

According to Fisher's formulas³

$$\sigma_\gamma^2 = \frac{-\frac{\partial^2 L}{\partial \alpha^2}}{\frac{\partial^2 L}{\partial \alpha^2} \frac{\partial^2 L}{\partial \gamma^2} - \left(\frac{\partial^2 L}{\partial \alpha \partial \gamma} \right)^2} = \frac{\frac{1}{n\alpha^2} \sum \frac{z_i^2 F_i'^2}{1 - F_i^2}}{\sum \frac{z_i^2 F_i'^2}{1 - F_i^2} \sum \frac{F_i'^2}{1 - F_i^2} - \left(\sum \frac{z_i F_i'^2}{1 - F_i^2} \right)^2}, \quad (5)$$

$$\sigma_\alpha^2 = \frac{-\frac{\partial^2 L}{\partial \gamma^2}}{\frac{\partial^2 L}{\partial \alpha^2} \frac{\partial^2 L}{\partial \gamma^2} - \left(\frac{\partial^2 L}{\partial \alpha \partial \gamma} \right)^2} = \frac{\frac{\alpha^2}{n} \sum \frac{F_i'^2}{1 - F_i^2}}{\sum \frac{z_i^2 F_i'^2}{1 - F_i^2} \sum \frac{F_i'^2}{1 - F_i^2} - \left(\sum \frac{z_i F_i'^2}{1 - F_i^2} \right)^2}. \quad (6)$$

It will be observed that in those cases in which the values of x or of $z = \alpha(x - \gamma)$ are so balanced that $\sum z F'^2 / (1 - F^2) = 0$ or in case α is known the weight of γ is

$$\frac{1}{\sigma_\gamma^2} = n\alpha^2 \sum \frac{F'^2}{1 - F^2}$$

and will be simplest if $F'^2 / (1 - F^2)$ is constant. This amounts to having $F(z) = \sin z$ so that there is no effect of the biological up to a certain minimum logarithmic dose $x = \gamma - \pi/2\alpha$ and that all animals are affected by the dose $x = \gamma + \pi/2\alpha$. The solution of the equations (1) and (2) is, however, not simple for $F(z) = \sin z$. On the other hand, the simplest case for the solution of those equations seem to be when $F' / (1 - F^2)$ is con-

stant, i.e., when $F(z) = \tanh z$ and we are working on the growth curve.

If we have determined which type of curve we shall use, the discussion of the precision of determination of γ or of the relative determination of α for different observed values of P_i would be from (4), (5); but if it be desired to compare the precisions that would be figured on the basis of the assumption of two different curve-types as underlying the biological phenomenon we should substitute for α its value in terms of the dilution spread c and the observed values of P_i . To discuss the precision for the general case of any observed values of P_i is impracticable, but for a discussion of the cases in which $F(z)$ is an odd function and there are three values of P_i which are symmetrically distributed as $P_1 = P$, $P_2 = 1/2$, $P_3 = 1 - P$ we may use the expressions

$$\frac{1}{\sigma_\gamma^2} = n \frac{\phi^2(1 - 2P)}{c^2} \left[F_{(0)}'^2 + \frac{F_{(z_3)}'^2}{2PQ} \right], \quad \frac{\alpha^2}{\sigma_\alpha^2} = n \frac{\phi^2(1 - 2P) F_{(z_3)}'^2}{2PQ},$$

where $z_3 = \phi(1 - 2P)$. For the five respective cases of the growth curve, probability integral curve, z , $\sin z$ and $z/\sqrt{1 + z^2}$, the results become

$$\frac{c^2}{n\sigma_\gamma^2} = [\tanh^{-1}(1 - 2P)]^2(1 + 8PQ),$$

$$V^2(1/2 - P) \left\{ 0.6366 + 2 \frac{\left[\frac{1}{\sqrt{2\pi}} e^{-1/2 V^2(1/2 - P)} \right]^2}{PQ} \right\}, (1 - 2P)^2 \left(1 + \frac{1}{2PQ} \right),$$

$$3[\sin^{-1}(1 - 2P)]^2, (1 - 2P)^2 \left(\frac{1}{4PQ} + 8PQ \right),$$

where ϕ is the function inverse to F and V is the normal variate taken from tables of the probability integral. And, further,

$$\frac{\alpha^2}{n\sigma_\alpha^2} = 8PQ[\tanh^{-1}(1 - 2P)]^2, V^2(1/2 - P) 2 \frac{\left[\frac{1}{\sqrt{2\pi}} e^{-1/2 V^2(1/2 - P)} \right]^2}{PQ},$$

$$\frac{(1 - 2P)^2}{2PQ}, \quad 2[\sin^{-1}(1 - 2P)]^2, \quad 8PQ(1 - 2P)^2.$$

From these expressions we may calculate tables 1 and 2. From table 1 it is clear that in the symmetrical case the values of σ_γ^2 for the growth curve and for the probability integral curve are very nearly the same for any value of P one would be likely to use. It is further clear that for other types of curve assumed as underlying the biological relationship the values of σ_γ may be very different from those found for either of them. It therefore cannot be assumed that σ_γ may not be markedly influenced by the

particular curve-type assumed. What curve-type should be used could only be settled by an extensive series of observations. Moreover, although the analysis has been carried out only for the symmetrical case, yet it seems

TABLE 1
VALUES OF $c^2/(n\sigma_\gamma^2)$ FOR CERTAIN VALUES OF P

	$P=0.40$	$P=0.20$	$P=0.10$	$P=0.05$	$P=0.01$	$P=0$
$F = \tanh z$	0.120	1.10	2.08	2.99	5.70	∞
$F = \frac{2}{\sqrt{2\pi}} \int_0^z e^{-z^2/2} dz$	0.120	1.15	2.17	2.93	4.22	∞
$F = z$	0.123	1.48	4.20	9.34	49.5	∞
$F = \sin z$	0.121	1.24	2.58	3.76	5.63	7.40
$F = z/\sqrt{1+z^2}$	0.118	1.02	2.24	4.57	24.3	∞

reasonable to assume that for cases likely to arise in practice, even though asymmetrical, the growth curve and probability integral curve would yield values of σ_γ more or less alike. The values found for γ itself might also be different in the asymmetrical case, though again it would be reasonable to assume that they would not differ much in ordinary cases.

If it is desired to standardize two biologicals it is of importance to determine whether the two values of α are nearly enough alike so that if the standardization is made at a pair of corresponding points such as the L.D.50 points, it may be regarded as valid for other points. As

$$\sigma\left(\log \frac{\alpha_2}{\alpha_1}\right) = \sqrt{\frac{\sigma_{\alpha_1}^2}{\alpha_1^2} + \frac{\sigma_{\alpha_2}^2}{\alpha_2^2}},$$

we may get some idea of the variation which would be involved in comparing both biologicals on the assumption of different curve-types as underlying the phenomenon, in cases where for each determination the distribution

TABLE 2
VALUES OF $\alpha^2/(n\sigma_\alpha^2)$ FOR CERTAIN VALUES OF P

	$P=0.40$	$P=0.20$	$P=0.10$	$P=0.05$	$P=0.01$	$P=0$
$F = \tanh z$	0.079	0.615	0.870	0.823	0.418	0
$F = \frac{2}{\sqrt{2\pi}} \int_0^z e^{-z^2/2} dz$	0.080	0.694	1.12	1.21	0.777	0
$F = z$	0.083	1.12	3.56	8.53	48.5	∞
$F = \sin z$	0.081	0.828	1.72	2.51	3.75	4.94
$F = z/\sqrt{1+z^2}$	0.077	0.461	0.461	0.308	0.076	0

is symmetrical. From table 2 it will be apparent that the precision of the relative determination of α is by no means so nearly the same for the growth curve and for the probability integral curve as was the case for the determination of γ , and that for other curves it may be very different.

¹ These PROCEEDINGS, 29, 79-85, 114-120 (1943).

² If only two dilutions are used and ϕ is the function inverse to F we should have at once

$$\phi(2P_1 - 1) = \alpha(x_1 - \gamma), \quad \phi(2P_2 - 1) = \alpha(x_2 - \gamma),$$

$$\alpha = \frac{\phi(2P_2 - 1) - \phi(2P_1 - 1)}{x_2 - x_1}, \quad \gamma = \frac{x_2 + x_1}{2} - \frac{x_2 - x_1}{2} \frac{\phi(2P_2 - 1) + \phi(2P_1 - 1)}{\phi(2P_2 - 1) - \phi(2P_1 - 1)},$$

and the general solution can be obtained whenever the inverse function ϕ may be found. For the respective cases $F = \tanh z$, $F = z$, $F = \sin z$, $F = z/\sqrt{1+z^2}$ the inverse functions are $\tanh^{-1} F$, F , $\sin^{-1} F$, $F/\sqrt{1-F^2}$, where F has the value $2P - 1$.

³ These formulas are those that would result from applying the method of differentiation in the usual manner.

$$\delta \frac{\partial L}{\partial \gamma} = - \sum \left[\frac{2s_i F_i'}{1 - F_i^2} - n \frac{F_i'}{1 - F_i^2} \right] \delta \alpha + \frac{\partial^2 L}{\partial \alpha \partial \gamma} \delta \alpha + \frac{\partial^2 L}{\partial \gamma^2} \delta \gamma - n \alpha \sum \frac{2F_i' \delta P_i}{1 - F_i^2} = 0.$$

The first term vanishes by virtue of equation (1) which permits the use of equations (3) and (4) for the second derivatives, it being understood that the actual values of s_i are to be considered as having arisen by variations from those on the fitted curve and that δP_i represents variations in the fitted values P_i from a curve with specified parameters α , γ to one with other parameters $\alpha + \delta \alpha$, $\gamma + \delta \gamma$. In this manner we obtain also

$$\delta \frac{\partial L}{\partial \alpha} = \frac{\partial^2 L}{\partial \alpha^2} \delta \alpha + \frac{\partial^2 L}{\partial \alpha \partial \gamma} \delta \gamma + n \sum \frac{2(x_i - \gamma) F_i' \delta P_i}{1 - F_i^2} = 0.$$

If H represents the Hessian determinant,

$$H \delta \gamma = 2n \sum \left[\alpha \frac{\partial^2 L}{\partial \alpha^2} + (x_i - \gamma) \frac{\partial^2 L}{\partial \alpha \partial \gamma} \right] \frac{F_i' \delta P_i}{1 - F_i^2}.$$

As the variations δP_i are considered as independent with standard deviation (squared) of $P_i Q_i / n = 1/4(1 - F_i^2)/n$ we have

$$H^2 \sigma_\gamma^2 = n \sum \left[\alpha^2 \left(\frac{\partial^2 L}{\partial \alpha^2} \right)^2 + 2\alpha(x_i - \gamma) \frac{\partial^2 L}{\partial \alpha^2} \frac{\partial^2 L}{\partial \alpha \partial \gamma} + (x_i - \gamma)^2 \left(\frac{\partial^2 L}{\partial \alpha \partial \gamma} \right)^2 \right] \frac{F_i'^2}{1 - F_i^2}.$$

Now substituting from equations (3) and (4) we have

$$H^2 \sigma_\gamma^2 = \left[- \left(\frac{\partial^2 L}{\partial \alpha^2} \right)^2 \frac{\partial^2 L}{\partial \gamma^2} + 2 \frac{\partial^2 L}{\partial \alpha^2} \left(\frac{\partial^2 L}{\partial \alpha \partial \gamma} \right)^2 - \left(\frac{\partial^2 L}{\partial \alpha \partial \gamma} \right)^2 \frac{\partial^2 L}{\partial \alpha^2} \right] = -H \frac{\partial^2 L}{\partial \alpha^2}$$

which gives the Fisher formula.

It may be noted that if we desire to obtain a numerical solution of equations (1) and (2) we may proceed by successive approximations. Provided we can find in some way values α_0 , γ_0 which nearly satisfy the equations, better values $\alpha_0 + \delta \alpha$, $\gamma_0 + \delta \gamma$ may usually be found from the equations

$$\left. \frac{\partial L}{\partial \gamma} \right|_0 + \left. \frac{\partial^2 L}{\partial \gamma^2} \right|_0 \delta \gamma + \left. \frac{\partial^2 L}{\partial \alpha \partial \gamma} \right|_0 \delta \alpha = 0, \quad \left. \frac{\partial L}{\partial \alpha} \right|_0 + \left. \frac{\partial^2 L}{\partial \alpha \partial \gamma} \right|_0 \delta \gamma + \left. \frac{\partial^2 L}{\partial \alpha^2} \right|_0 \delta \alpha = 0.$$

RELATIONS BETWEEN HOMOLOGY AND HOMOTOPY GROUPS

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1. *The Problem.*—Let P be a locally finite simplicial connected polytope. We denote by $\mathfrak{H}^q(P, G)$ the (discrete) q th homology group of the finite chains of P over a (discrete additive) coefficient group G , by $H_q(P, G)$ the (topologized)¹ q th cohomology group of the infinite cochains of P over a (topologized additive) coefficient group G , by $\pi_q(P)$ the q th homotopy group of P relative to a fixed base point $x_0 \in P$, by $\mathfrak{S}^q(P, G)$ the subgroup of $\mathfrak{H}^q(P, G)$ determined by the cycles of P that can be obtained from cycles on the q -sphere S^q by continuous mappings of S^q into P .

H. Hopf² has shown that the fundamental group $\pi_1(P)$ determines the group $\mathfrak{H}^2(P, I)/\mathfrak{S}^2(P, I)$ (I = additive group of all integers) and has exhibited a group construction which leads from π_1 to $\mathfrak{H}^2/\mathfrak{S}^2$. He considers a multiplicative discrete group $\pi = F/R$ represented as a factor group of a free group F by a group of relations R and defines

$$\pi_1^* = R \cap \text{Com } F / \text{Com}_R R, \quad (1.1)$$

where $\text{Com } F$ stand for the commutator group of F , $\text{Com}_R R$ is the subgroup of F generated by the elements of the form $xrx^{-1}r^{-1}$, $x \in F$, $r \in R$, while “ \cap ” stands for the intersection.

Hopf shows that the group π_1^* does not depend upon the particular representation chosen for π and that if $\pi = \pi_1(P)$ then $\pi_1^* \cong \mathfrak{H}^2(P, I)/\mathfrak{S}^2(P, I)$.

In a more recent paper³ Hopf has shown that if $\pi_i(P) = 0$ for all $1 < i < n$ then $\pi_1(P)$ determines the group $\mathfrak{H}^n(P, I)/\mathfrak{S}^n(P, I)$. This proof leads to no method for the algebraic determination of $\mathfrak{H}^n/\mathfrak{S}^n$ by means of π_1 .

We outline here a new treatment of the problem which leads to generalizations of Hopf's results and gives intrinsic descriptions for all the groups involved. In particular the group π_1^* can be defined intrinsically as the character group⁴ of the group of central group extensions⁵ of the group X (reals reduced mod 1) by the group π :

$$\pi_1^* = \text{Char Extcent } \{X, \pi\} \quad (1.2)$$

The groups obtained for the higher cases may rightly be regarded as generalizations of the group of group extensions.

The method used also permits us to generalize and simplify the results of Hopf dealing with the influence of $\pi_1(P)$ upon the intersection theory in P .

The results are formulated for a connected polytope P but are valid for any arcwise connected space, provided suitable singular homologies are used.

2. *The Complex $K(\pi)$.*—Given a discrete group π written multiplicatively we will consider square matrices $\Delta = \|p_{ij}\|$ with p_{ij} in π satisfying the condition

$$p_{ij}p_{jk} = p_{ik}. \quad (2.1)$$

We will denote by $\Delta^{(i)}$ the matrix obtained from Δ by erasing the i th row and the i th column. The rows and columns will always be numbered starting from 0. The matrices Δ with $q + 1$ rows and columns will be taken as the generators of a free abelian group C^q . We define a homomorphism

$$\alpha: C^q \rightarrow C^{q-1}$$

by putting

$$\alpha \Delta = \sum_{i=0}^q (-1)^i \Delta^{(i)}$$

for each generator Δ of C^q . We verify that $\alpha\alpha = 0$. Consequently if we consider each generator Δ of C^q as a q -cell and α as a boundary operation we obtain a closure finite abstract complex $K(\pi)$. The (discrete) q th homology group obtained by using finite chains of $K(\pi)$ over a (discrete) coefficient group G will be written as $\mathfrak{H}^q(\pi, G)$. The (topologized) q th cohomology group obtained using infinite cochains of $K(\pi)$ over a (topologized) coefficient group G will be written as $H^q(\pi, G)$.

3. *The Main Theorem.*—Let s^q be q -dimensional simplex with vertices v_0, v_1, \dots, v_q and let $T: s^q \rightarrow P$ be a continuous mapping of s^q into the polytope P such that the vertices v_i are all mapped into the point x_0 in P (here x_0 is the base point for the construction of the fundamental group $\pi_1 = \pi_1(P)$). Each edge $v_i v_j$ in s^q will then determine an element p_{ij} in the group π_1 and the matrix $\|p_{ij}\|$ so obtained will satisfy condition (2.1). Hence the mapping T determines a q -cell of the complex $K(\pi_1)$. This observation leads to a chain transformation

$$\tau: P \rightarrow K(\pi_1(P)) \quad (3.1)$$

A study of this transformation furnishes the following:

THEOREM 1. *If a connected locally finite polytope P has*

$$\pi_i(P) = 0 \quad \text{for } 1 < i < n, \quad (3.2)$$

the following isomorphisms hold:

$$\mathfrak{H}^q(P, G) \cong \mathfrak{H}^q(\pi_1(P), G) \quad \text{for } q < n, \quad (3.3)$$

$$H_q(P, G) \cong H_q(\pi_1(P), G) \quad \text{for } q < n, \quad (3.4)$$

$$\mathfrak{H}^n(P, G)/\mathfrak{H}^n(P, G) \cong \mathfrak{H}^n(\pi_1(P), G). \quad (3.5)$$

4. *Computation of the Groups $H_q(\pi, G)$.*—We will outline a method for computing the groups $\tilde{H}^q(\pi, G)$ and $H_q(\pi, G)$ directly in terms of π and G without using the complex $K(\pi)$. It is sufficient to compute the cohomology groups $H_q(\pi, G)$, since the homology-cohomology duality applied to the complex $K(\pi)$ gives

$$\tilde{H}^q(\pi, G) \cong \text{Char } H_q(\pi, \text{Char } G), \quad G \text{ discrete.}$$

Our calculations are based upon a 1 — 1 correspondence between the matrices Δ with elements in π and the q -tuples (p_1, \dots, p_q) with $p_i \in \pi$. Given such a q -tuple, the definitions $p_{ii} = 1$, $p_{ij} = p_i + 1 \dots p_j$ for $i < j$ and $p_{ij} = (p_{ji})^{-1}$ for $j < i$ define a $q + 1$ by $q + 1$ matrix $\Delta = \|p_{ij}\|$ which satisfies (2.1). Conversely, every such matrix Δ can be obtained in this way from exactly one q -tuple. Consequently the group of q -cochains of the complex $K(\pi)$ over the (topologized) group G is nothing but the group $C_q(\pi, G)$ of all functions f of q variables on π to G . The coboundary δf of such a function is a function of $q + 1$ variables defined as follows

$$(\delta f)(p_1, p_2, \dots, p_{q+1}) = f(p_2, \dots, p_{q+1}) + \sum_{i=1}^q (-1)^i f(p_1, \dots, p_{i-1}, p_i p_{i+1}, p_{i+2}, \dots, p_{q+1}) + (-1)^{q+1} f(p_1, p_2, \dots, p_q).$$

The group $Z_q(\pi, G)$ of cocycles consists of the functions f with $\delta f = 0$, while the group $B_q(\pi, G)$ of the cobounding cocycles consists of those functions f of the form $f = \delta g$. The cohomology groups are

$$H_q(\pi, G) = Z_q(\pi, G) / B_q(\pi, G).$$

5. *The Cases $q = 1, 2, 3$.*—The coboundary of a function of one variable $f: \pi \rightarrow G$ is

$$(\delta f)(p_1, p_2) = f(p_2) - f(p_1 p_2) + f(p_1). \quad (5.1)$$

Hence $Z_1(\pi, G)$ is composed of the homomorphisms $f: \pi \rightarrow G$. Since $B_1(\pi, G) = 0$ we have

$$H_1(\pi, G) = \text{Hom } \{\pi, G\}.$$

For a function f of two variables the coboundary is

$$(\delta f)(p_1, p_2, p_3) = f(p_2, p_3) - f(p_1 p_2, p_3) + f(p_1, p_2) - f(p_1, p_3). \quad (5.2)$$

The condition that $\delta f = 0$ is exactly the associativity condition for a "central" factor set $f(p_1, p_2)$ of π in G , while the coboundaries δf in (5.1) of functions of one variable are simply the "central" transformation sets. Hence $H_2(\pi, G)$ is the group of factor sets modulo transformation sets, and so is the group of central extensions of G by π ,

$$H_2(\pi, G) \cong \text{Extcent } \{G, \pi\}.$$

For $q = 3$ the coboundary δf of f becomes

$$(\delta f)(p_1, p_2, p_3, p_4) = f(p_2, p_3, p_4) - f(p_1 p_2, p_3, p_4) + f(p_1, p_2 p_3, p_4) \\ - f(p_1, p_2, p_3 p_4) + f(p_1, p_2, p_3). \quad (5.3)$$

This operation and the resulting group $H_3(\pi, G)$ have been defined by O. Teichmüller,⁶ in a somewhat more general case (corresponding to group extensions which are not necessarily central).

6. *Higher Dimensions.*—A generalization of the main theorem to higher dimensions can be obtained by constructing for each discrete *abelian* group π and each integer $n > 0$ a complex $K(\pi, n)$ whose cells are suitable systems of elements in π with $n + 1$ indices. Denoting the homology and cohomology groups of $K(\pi, n)$ by $\mathfrak{H}^q(\pi, n, G)$ and $H_q(\pi, n, G)$ we have

Theorem 2.—Given a connected locally finite polytope P such that

$$\pi_i(P) = 0 \quad \text{for } 0 < i < m \quad \text{and for } m < i < n \quad (6.1)$$

the following isomorphisms hold:

$$\mathfrak{H}^q(P, G) \cong \mathfrak{H}^q(\pi_m(P), m, G), \quad q < n, \quad (6.2)$$

$$H_q(P, G) \cong H_q(\pi_m(P), m, G), \quad q < n, \quad (6.3)$$

$$\mathfrak{H}^n(P, G)/\mathfrak{S}^n(P, G) \cong \mathfrak{H}^n(\pi_m(P), m, G). \quad (6.4)$$

¹ The topological groups are understood in a generalized sense, with the Hausdorff separation axiom not postulated.

² *Commentarii Math. Helvetici*, **14**, 257–309 (1942).

³ *Ibid.*, **15**, 27–32 (1942).

⁴ The group *Char* G of a given abelian group G is the suitably topologized group of all homomorphisms of the group G into the group X of reals reduced mod 1.

⁵ A group E is a central extension of X by H if X is a normal subgroup of E which lies in the center of E and has $E/X = H$. The group of group extensions (including non-central cases) is treated in H. Zassenhaus, *Lehrbuch der Gruppentheorie*, Hamburg Math. Einzelschriften, Leipzig 21, 1937.

⁶ O. Teichmüller, "Ueber die sogenannte nichtkommutative Galoische Theorie und die Relation $\xi_{\lambda, \mu} \xi_{\lambda, \mu}, \pi \xi_{\mu, \nu}, \pi^\lambda = \xi_{\lambda, \mu, \nu} \xi_{\lambda \mu, \nu}, \pi$." *Deutsche Math.*, **5**, 138–149 (1940).

A STRESS FUNCTION FOR THE MEMBRANE THEORY OF SHELLS OF REVOLUTION

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1. *The Membrane Theory.*—The partial differential equations satisfied by the stresses in a problem concerning the equilibrium of a loaded thin shell or dome of the form of a surface of revolution are very much simplified if it is assumed that the state of stress is independent of any displacements that may occur. The resulting treatment is called "The Membrane Theory." The equations have long been known but seldom solved except in simple cases. The special case when the loads and supports on every meridian of a tank or a dome are the same is solved in the standard engineering texts; the case of a dome loaded or supported non-uniformly has hitherto been solved exactly only for a few surfaces generated by conics, although some approximate methods have been suggested for other cases. If problems of non-uniform support or non-symmetric load are to be treated, it must be within the membrane theory, for the partial differential equations of the ordinary infinitesimal or "bending" theory are so complicated as to render their solution in most cases impracticable.

In this note we produce a simple method of exact solution of the membrane equations for any surface of revolution and for any load representable by a Fourier polynomial; for several families of surfaces of particular interest, we list the solutions explicitly.

2. *The Stress Function.*—If ϕ is the angle between the normal to the surface and the axis of revolution, θ the angle of rotation measured from some given plane through the axis, R_1 and R_2 the radii of normal curvature, N_ϕ and N_θ the tangential and meridial stresses, $N_{\theta\phi}$ the shear, X , Y , Z , respectively, the parallel, meridial and normal components of the load, then the membrane equations for a shell having the form of a surface of revolution are¹

$$\left. \begin{aligned} \frac{N_\phi}{R_1} + \frac{N_\theta}{R_2} &= -Z, \\ \frac{\partial}{\partial \phi}(N_\phi R_2 \sin \phi) - N_\theta R_1 \cos \phi + R_1 \frac{\partial N_{\theta\phi}}{\partial \theta} + Y R_1 R_2 \sin \phi &= 0, \\ R_1 \frac{\partial N_\theta}{\partial \theta} + N_{\theta\phi} R_1 \cos \phi + \frac{\partial}{\partial \phi}(N_{\theta\phi} R_2 \sin \phi) + X R_1 R_2 \sin \phi &= 0. \end{aligned} \right\} \quad (1)$$

We shall suppose the stresses and loads represented as Fourier polynomials:

$$\left. \begin{aligned} X &= \sum_{n=0}^p X_n(\phi) \sin n\theta, & Y &= \sum_{n=0}^p Y_n(\phi) \cos n\theta, & Z &= \sum_{n=0}^p Z_n(\phi) \cos n\theta, \\ N_\phi &= \sum_{n=0}^p N_{\phi n}(\phi) \cos n\theta, & N_{\theta\phi} &= \sum_{n=0}^p N_{\theta\phi n}(\phi) \sin n\theta, & N_\theta &= \sum_{n=0}^p N_{\theta n}(\phi) \cos n\theta. \end{aligned} \right\} \quad (2)$$

Now let the equation of the generating curve in the $\theta = 0$ plane be $r = f(z)$, where z is measured along the axis of revolution, and let

$$U_n(\phi) \equiv R_2 f(z) N_{\phi n} \sin^2 \phi. \quad (3)$$

If we substitute (2) and (3) in (1), taking advantage of the geometry of the surface, and eliminate $N_{\theta n}$ and $N_{\theta\phi n}$, we are led to the differential equation of the stress function U_n :

$$\frac{d^2 U_n}{dz^2} + (n^2 - 1) \frac{f''}{f} U_n = - \frac{d}{dz} [f^2 f' Z_n] + f[1 + (n^2 - 1)(1 + f'^2)] Z_n + f^2 \frac{dY_n}{dz} + 3ff' Y_n + nX_n f \sqrt{1 + f'^2}. \quad (4)$$

When there is no load, the right side vanishes; the equation (4) for this case has been derived previously by Nemenyi from graphostatic considerations.² Once the solution of (4) has been obtained for a given surface f , the coefficients of (2) may be found from the formulas

$$\left. \begin{aligned} N_{\phi n} &= \frac{\sqrt{1 + f'^2}}{f^2} U_n, \\ N_{\theta n} &= \frac{f''}{f \sqrt{1 + f'^2}} U_n - f \sqrt{1 + f'^2} Z_n, \\ nN_{\theta\phi n} &= - \frac{d}{dz} \left(\frac{U_n}{f} \right) + f(f' Z_n - Y_n). \end{aligned} \right\} \quad (5)$$

Since the method of solution of (4) is well known, we may now consider (1) as solved for all cases when expansions of type (2) are justified. Since, however, to our knowledge there are very few known solutions of (1),³ it is interesting to write down the explicit solution of (4) for a few surfaces. We shall refer to solutions of (4) with right side zero as "homogeneous solutions"; from them the general solutions of (4) can easily be found.

3. *A Few Solutions.*—If the equation of the surface f satisfies a differential equation

$$\frac{f''(z)}{f(z)} = A^2 p(z) \quad (6)$$

for some function $p(z)$, then the homogeneous solutions for f can be obtained by putting $-A^2(n^2 - 1)$ for A^2 in the integral of (6), due care being taken to adjust the solutions when this change of constant produces or destroys an integer exponent difference. The indirect method of solving (4) by choosing a function $p(z)$ such that (6) is easily integrable has enabled us to find numerous infinite families of surfaces for which (1) can be solved exactly and in finite form. We list a few of those of possible importance for the engineering applications.

$$\left. \begin{aligned} f &= \sqrt{a-z} \sqrt{b-z} \left[R + S \log \frac{a-z}{b-z} \right], \\ U_n &= \sqrt{a-z} \sqrt{b-z} \left[A_n \left(\frac{b-z}{a-z} \right)^{n/2} + B_n \left(\frac{a-z}{b-z} \right)^{n/2} \right]. \end{aligned} \right\} \quad (7)$$

$$\left. \begin{aligned} f &= R(z+a)^p + S(z+a)^{1-p}, \quad p \neq 1/2 \\ f &= \sqrt{z+a} [R + S \log(z+a)], \quad p = 1/2 \\ U_n &= \sqrt{z+a} [A_n (z+a)^{q_n} + B_n (z+a)^{-q_n}], \end{aligned} \right\} \quad (8)$$

where $q_n^2 \equiv 1/4 - p(p-1)(n^2-1)$.

$$\left. \begin{aligned} f &= \sqrt{z+a} B_m(2mA[z+a]^{1/2m}), \quad m \neq 0, \\ U_n &= \sqrt{z+a} B_m(2miA \sqrt{n^2-1} [z+a]^{1/2m}), \end{aligned} \right\} \quad (9)$$

where $B_m(x)$ is the complete solution of Bessel's equation of order m .

The case $S = 0$, $b = -a$ in (7) gives us, by choice of R , a sphere, a spheroid or a hyperboloid of two sheets; in the first case, the homogeneous solutions reduce to the known solution for the sphere.⁴ If we put ia for a , we have a hyperboloid of one sheet. The case $p = 1/2$ of (8) is a paraboloid. The case $m = 1/2$ of (9) gives us a surface generated by the ordinary circular or hyperbolic functions. It is interesting to notice that the solutions for a logarithmic surface are not essentially different from those for an algebraic one; e.g., U_2 for $f = \sqrt{z+a} \log(z+a)$ is the same as U_3 for

$$f = (z+a)^{1/2(1+\sqrt{3}/2)}.$$

We have found the solutions for many more surfaces, including those for any surface generated by any solution of the second order equation with two regular singularities, or by any confluent hypergeometric function. There are now enough harmonics found explicitly that it is reasonably easy

to obtain a good approximation to the solution of any soluble statically determinate membrane problem.

4. *Subsequent Results.*—A subsequent investigation by Truesdell alone has produced the following results:

(a) A rigorous deduction of an equation similar to (4) whenever the loads can be represented by Fourier series.

(b) A complete direct solution of (1) for the case $n = 1$, which is of considerable interest in problems of wind-pressure.

(c) The solution of several specimen boundary problems of spheroids, paraboloids and hyperboloids, and a simple method of solution of a dome of any cross-section supported in any manner, and having a lantern bearing a specified load in any manner.

(d) A general theorem on the character of the zeros of U_n as related to the zeros of f ; a corollary showing that the problem of a closed dome with a zero of order μ at the apex and supported in any non-uniform manner is not soluble within the membrane theory if $\mu \geq 1$, is soluble if $1/2 < \mu < 1$ if, and only if, the supports are symmetrically placed and are large enough in number, and is always soluble for two or more symmetrical supports if

$$0 < \mu \leq 1/2.$$

* The work outlined in sections 1 to 3 of this note was done while the authors were fellows at the School of Mathematical Mechanics, Brown University.

¹ See Reissner, H., *Spannungen in Kugelschalen (Kuppeln)*, Festschrift Heinrich Müller Breslau gewidmet, Leipzig, 1912, pp. 181–193, and Flügge, W., *Statik und Dynamik der Schalen*, Berlin 1934, pp. 23–24.

² P. Nemenyi, *Beiträge zur Berechnung der Schalen unter unsymmetrischer und un-stetiger Belastung*, Byggningsstatiska Meddelelser, 1936.

³ Flügge, op. cit., Chap. II.

⁴ Ibid., p. 39.

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*ELECTRICAL CORRELATES OF PURE AND HYBRID STRAINS OF SWEET CORN**

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It has been said by numerous investigators that the key problem in biology is organization. The patterns which living organisms present form the basis of description and classification in natural history. Adequate as these are in many instances, little evidence has been forthcoming concerning the forces which impose this stable and characteristic pattern on the dynamic flux which is unique in living systems. The geneticists have been extraordinarily successful in showing that many of the properties of the pattern can be related to structural elements in the chromosomes, including even molecular linkages. The mechanism by means of which the genes control form is still hidden. It is at this point that the key problem, the pattern of organization, arises.

The search for an answer to the problem has fallen rather naturally into three channels. In the first, the investigations have tended to assume that the forces operating were peculiar to biological systems. This has led to a number of fruitful qualitative descriptions such as physiological gradient, biological and embryological fields. These terms include no quantitative measures and, therefore, cannot be genuinely rigorous. The most popular approach has been through chemistry. The relationship between morphogenesis and chemical entities and processes has been widely examined. Out of such studies has come a series of descriptions of the building blocks of which protoplasm is composed, but so far it has failed to provide significant evidence of how, in the midst of constant chemical change, the design remains so astonishingly constant. The third channel is still relatively unexplored. A few sporadic and still unsuccessful attempts have been made to influence the shape of living things through physical force. However, the development of modern techniques of electrical measurement has made it possible to explore the electrical correlates of this design more effectively.

In 1932 Burr,¹ and in 1935 Burr and Northrop,² proposed a working hypothesis to the effect that the electrical signs to be found everywhere among living things indicate the existence of an underlying electrodynamic field whose characteristic forces impose pattern on protoplasm. In the past seven or eight years, a number of studies coming from Burr's laboratory have tended to support the validity of the working hypothesis. More particularly, in 1941³ he showed that in the developing frog's egg a characteristic electrical pattern could be determined in the unfertilized egg, and in the fertilized egg before the advent of the characteristic form of the embryo. The axis of this electrical pattern was found to predict the longitudinal axis of the nervous system. These findings suggest that there is a close relationship between the electrical pattern and the bilateral symmetry of the embryo. Genetics has shown the structural pattern to be closely associated with chromosomal arrangement. It follows, therefore, that in all probability, there is a very close relationship between electrical pattern and the genetic constitution of the living organism. To investigate this problem is difficult in the animal kingdom. In the plant world, however, the situation is simpler for genetic controls are more readily imposed and the consequences more readily observed.

With the coöperation of the authorities of the Connecticut Agricultural Experiment Station, it has been possible to study the electrical patterns in several pure and hybrid strains of sweet corn. The corn seeds used were from strains which have been under study for some time. These strains differ considerably in genetic constitution and in the degree of hybrid vigor shown in crosses between them.

The seeds were collected in 1942 and studied during the winter of 1942-1943. Four inbred strains were studied, and three hybrids. The inbreds used were C6, C13, P39, and a mutant of P39, C30. The hybrids were P39 \times C13, C30 \times C13, and C6 \times C13. C6 is the seed parent of Whip-cross C6.2, with ears set low on stalk and well formed. It is resistant to bacterial wilt and rust. C13 is a Golden Early Market inbred. It is used as the seed or parent pollen of Marcross, C13.6. It is highly resistant to bacterial wilt. P39 is a mid-season yellow sweet corn, inbred, of unknown origin. C30 is a semi-dwarf mutant of P39. It is normal in appearance though much reduced in size. It is completely recessive to the P39. It has been shown by Singleton⁴ to differ by only a single gene from P39.

The hybrids studied have shown considerable differences in hybrid vigor in the field. When P39 is crossed with C13, the average weight of the ears is significantly less than in the hybrid C30 \times C13. This was true in 1940 and again in 1942. In 1941, however, there was no difference. In spite of the dwarfism of C30, the hybrids from this line seem to have slightly more hybrid vigor than P39 hybrids. The hybrid C6 \times C13, known as "Marcross," is still the outstanding early season hybrid (Singleton, personal com-

munication). In this material, therefore, there are available four stable pure strains of significantly different properties with which to correlate electrical patterns. The three hybrids between them show gradation of hybrid vigor which is least in $P39 \times C13$ and greatest in $C6 \times C13$. If electrical patterns have any significance, electrical correlates of these differences should be manifest.

The technique employed was standard, consisting of the determination of a standing potential between the two opposite ends of the longitudinal axis of the kernel. The point of the attachment of the kernel to the cob was invariably positive and the opposite pole negative. Contact with the system was made by silver-silver chloride electrodes and measurements made with a microvoltmeter and galvanometer. More than 2000 measurements were made under carefully controlled conditions with the results shown in table 1.

A statistical analysis[†] shows that the F factor is 6.4. The requirement for 1% significance is 3.07. It is safe to conclude, therefore, that the means of all the strains differ significantly from each other. A second set of determinations was carried out using a larger number of kernels but with a smaller number of measurements for each kernel, and the means were found to check closely with those shown in table 1. Aside from the generally different means the most striking finding was the very great difference between the mean of the single gene mutant $C30$ and the parent stock $P39$. It is remarkable that the change of a single gene in $P39$ should produce such a profound and significant change in the over-all pattern of voltage difference.

TABLE I
STATISTICAL ANALYSIS OF POTENTIAL DIFFERENCES IN SWEET CORN SEEDS

Means in millivolts						
C30	PURE STRAINS		P39	$P39 \times C13$	HYBRIDS	
	C13	C6			$C30 \times C13$	$C6 \times C13$
6.2	19.4	23.8	24.05	14.5	17.4	23.3
$F = 6.4$. 1% Sig., 3.07. 5% Sig., 2.23.						

The conclusion seems to be inescapable, that there is a very close relationship between the genetic constitution and the electrical pattern. If further studies should confirm this conclusion, it seems very probable that one of the ways the chromosomes impart design to protoplasm is through the medium of an electrodynamic field.

No less interesting than the electrical correlates of the pure strains is the relationship between the potential differences and hybrid vigor. The electrical studies show a significant relationship between the potential difference and the degree of hybrid vigor, a relatively high potential difference

being found in association with a high degree of hybrid vigor in the field; and a lower difference with a lower degree of vigor.

The search for a significant measure of hybrid vigor in plants has yielded confusing and inconclusive evidence. The findings here reported suggest that by means of the measured potential difference it may be possible to predict hybrid vigor.

Summary.—1. Electrical correlates have been found between different inbred strains of sweet corn even when the difference is due only to a single gene.

2. The magnitude of the potential difference is positively correlated with the degree of hybrid vigor.

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† The aid of Dr. Chester I. Bliss and Professor I. L. Child is gratefully acknowledged.

¹ Burr, H. S., *Jour. Comp. Neur.*, 56, No. 2, 347–371, Dec. 15 (1932).

² Burr, H. S., and Northrop, F. S. C., *Quart. Rev. Biol.*, 10, No. 3, 322–333, Sept. (1935).

³ Burr, H. S., *Proc. Nat. Acad. Sci.*, 27, No. 6, 276–281, June (1941).

⁴ Singleton, W. R., *Genetics*, 28, No. 1, 89 (1943).

SYNTHESIS OF RIBOFLAVIN BY A YEAST

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Previous investigations have shown that yeasts require one or more members of the vitamin B complex in order to grow well in chemically defined media. It appears, however, that riboflavin (vitamin B₂) is not needed as a constituent of the culture media supporting yeast growth. Since riboflavin is known to play an important rôle as a respiratory enzyme generally throughout the plant and animal kingdoms, it seems probable that yeasts are able to synthesize this vitamin in amounts sufficient at least for their own requirements. In a preliminary survey of the production of vitamin B₂ by some 200 kinds of yeasts, one species has been found to produce extraordinary amounts of riboflavin in synthetic culture media.* This yeast offers, therefore, certain obvious advantages for studies on vitamin synthesis, and assumes special significance in relation to the commercial production of vitamin B₂ for use in animal and human nutrition. A brief account will be given concerning the influence of various factors, such as temperature, aeration, cyanide, composition of the medium, etc., upon the yield of riboflavin. The results of other experiments, including the

feeding of the yeast to animals for the purpose of determining possible toxicity, will be published elsewhere.

The yeast which has been employed for most of the work reported herewith was received as an unidentified species number 488 from Dr. Lynferd J. Wickerham, who isolated it from sour milk some six years ago in the Yale laboratories. Additional strains of yeasts which appear to belong in the same species were isolated from various sources and supplied to us by Dr. Wickerham. These organisms were found to produce riboflavin in varying amounts, indicating that appreciable biochemical variation exists among different strains of the yeast.

When the yeast was cultivated in mineral salts-glucose solution enriched with asparagine and biotin, the resulting fermented liquor possessed a deep yellow color not unlike that of synthetic riboflavin solutions. Upon irradiation with ultra-violet, the fermented liquor exhibited a green fluorescence. Further fluorometric determinations performed upon the eluate from florisil, and microbiological assays made on the cultures with the test organism *Lactobacillus casei* indicated the presence of large amounts of riboflavin in the fermented liquor. Separation of the yeast from the fermented medium by means of filtration or centrifugation yielded a clear yellow solution, and a mass of almost white yeast cells. Microbiological assays further indicated that nearly all the vitamin B₂ accumulates outside the yeast in the fermented liquor, thus eliminating the necessity for extracting the vitamin from the yeast by means of expensive solvents. Although riboflavin was produced by all strains of the species tested, strain designated No. 488 was found to be particularly efficient.

Composition of the Medium.—The culture solution employed in this study, unless stated otherwise, had the following composition per liter: KH₂PO₄, 0.5 g.; MgSO₄·7H₂O, 0.5 g.; CaCl₂·2H₂O, 0.3 g.; (NH₄)₂SO₄, 2.0 g.; KI, 0.1 mg.; dextrose, 20 g.; asparagine, 2.0 g.; biotin methyl ester, 1.0 µg. Trace elements were added in p. p. m. as follows: B, 0.01; Mn, 0.01; Zn, 0.07; Cu, 0.01; Mo, 0.01; and Fe, 0.05. Distilled water was employed in making the medium. The reaction of the culture solution was adjusted to pH 5.0. The medium was dispensed usually in the amount of 25 ml. into each 125-ml. Erlenmeyer flask. The flasks were plugged with cotton and sterilized by autoclaving at 120°C. for 15 minutes. After cooling, inoculation was accomplished by adding a drop of yeast suspension to each flask.

Variations in or omission of certain constituents of the medium, and the influence of substituting or adding other compounds were studied in relation to growth and the production of riboflavin. After incubating usually at 30°C. for about one week, growth of the yeast cultures was measured turbidimetrically, with appropriate corrections being made for the colored solutions. Since the amounts of the vitamin produced were relatively

great, it was found convenient to make quantitative determinations by absorption photometry. The yeast was removed from the cultures by filtration through supercel in a Büchner funnel. Corrections for absorption resulting from the presence of pigments other than riboflavin in the medium were made with the aid of uninoculated controls. Absorption of blue light by the solutions was determined with a Klett photoelectric colorimeter and the resulting values were then calculated in terms of vitamin B₂ by reference to a standard curve constructed for solutions of synthetic riboflavin. Microbiological assays gave good agreement with the photometric determinations.

The Source of Carbon.—A very important factor in the production of riboflavin is the source of carbon for fermentation. It appears that certain kinds of carbon compounds supplied in the medium may be used for the building of protoplasm but remain unavailable for synthesis of riboflavin. Certain sugars, such as Pfanstiehl's C.P. maltose and *d*-galactose, produce excellent growth, but yield only small amounts of riboflavin. Sucrose, dextrose and levulose promote both growth and production of vitamin B₂. When *d*-galactose was mixed with dextrose in the medium, excellent growth resulted, but only low yields of riboflavin were observed.

The kind of hexose sugar as well as its concentration in the medium appear to have a direct influence upon the production of pentose for incorporation into the riboflavin molecule. The influence of different concentrations of dextrose upon growth and yield of vitamin B₂ is shown graphically in figure 1. The experimental results presented in figure 1 were obtained with glycine and asparagine each present in the amount of 0.5 g. per liter of basal medium. Growth was allowed to proceed at 30°C. for a period of six days.

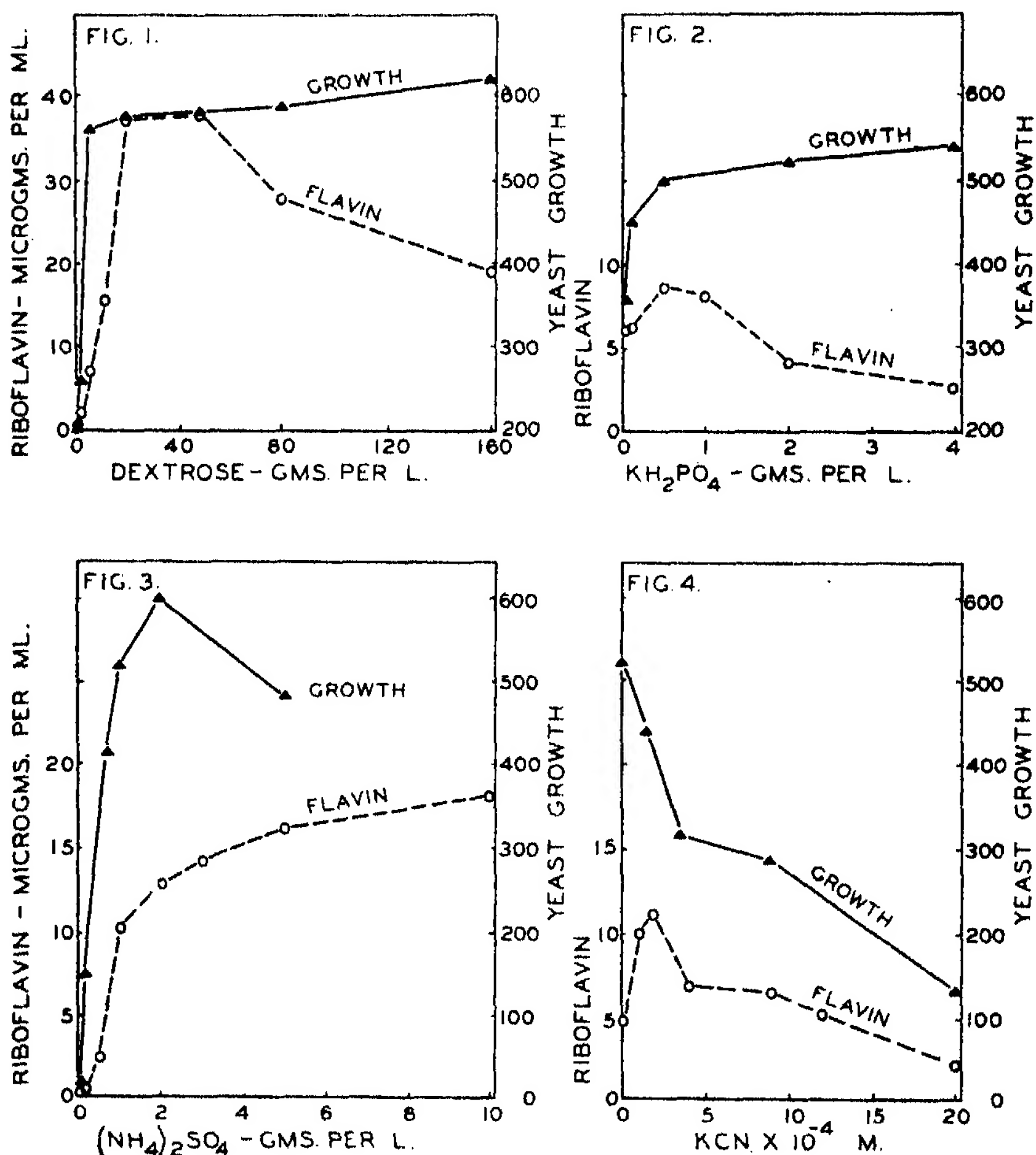
The Source of Nitrogen.—A suitable source of organic nitrogen seems to be of considerable importance for the formation of vitamin B₂. Appreciable amounts of the vitamin accumulated during fermentation of mineral-dextrose solutions containing nitrogen supplied only in inorganic form as (NH₄)₂SO₄. The addition of certain amino acids such as glycine, or amides such as asparagine served to augment the yield of riboflavin, without necessarily affecting growth of the yeast. Asparagine appeared to be more efficient than glycine for the production of vitamin B₂. A favorable medium may contain 0.5 g. of glycine and 0.5 g. of asparagine in addition to the usual 2.0 g. of (NH₄)₂SO₄ in 1 liter of the basal medium. Whereas, the yield of riboflavin varied with the increase of glycine and asparagine over a considerable range, production of the vitamin was decreased by adding hydrolyzed casein in amounts from 0.5 g. to 4.0 g. per liter. The growth obtained with supplements of casein, however, was at least equal to that observed in media containing glycine and asparagine. Other amino acids supplied in addition to asparagine may have little effect

upon yield, or in some instances may greatly enhance the production of riboflavin. When amino acids were added singly at the rate of 0.8 g. per liter along with asparagine occurring as 2.0 g. per liter in the medium the effectiveness for increasing the yield was as follows in ascending series: serine < aspartic acid < arginine < leucine < valine < glycine < methionine. Under comparable conditions the production of riboflavin in micrograms per ml. of fermented liquor was as follows: a medium containing N only as $(\text{NH}_4)_2\text{SO}_4$ in the amount of 2.0 g. per liter yielded 12.5 γ B_2 per ml.; with asparagine added in the amount of 2.0 g. per liter, 18.0 γ B_2 per ml. were produced; with asparagine 2.0 g. per liter plus glycine 0.8 g. per liter, 47.0 γ B_2 per ml.; and with asparagine 2.0 g. per liter plus methionine 0.8 g. per liter, 60.0 γ B_2 per liter were observed. It is of interest to point out that both asparagine and glycine are relatively efficient and comparatively inexpensive sources of organic nitrogen. In the experiment where 2.0 g. of asparagine and 0.8 g. of glycine per liter were employed, and fermentation allowed to proceed at 30°C. for about six days, the fermented liquor was separated from the yeast cells by filtration. Dehydration of the fermented liquor yielded a yellow powder which contained 7 mg. of riboflavin per gram of solids.

Inorganic Compounds.—Variations in the amounts of inorganic materials present in the medium were found to influence both growth and riboflavin content of the fermented liquor. In one experimental series using 50 ml. of medium and 2.0 g. of asparagine per liter and a growing temperature of 27°C., the supply of KH_2PO_4 in the nutrient solution was varied over a wide range. As shown in figure 2, riboflavin reached a maximum with KH_2PO_4 supplied at levels of about 0.5 to 1.0 g. per liter, and decreased with greater doses of the salt. Growth of the yeast rose markedly with increased amounts of KH_2PO_4 in the lower range, and was maintained for all values of the salt employed up to 4.0 g. per liter.

The influence of increasing the supply of $(\text{NH}_4)_2\text{SO}_4$ upon growth and yield of riboflavin in 50-ml. cultures growing at 27°C. is shown in figure 3. Growth increased sharply with increments of ammonium salt up to about 2.0 g. per liter. Above this level some decrease in growth was apparent. The formation of riboflavin in the fermented liquor was augmented greatly by increasing $(\text{NH}_4)_2\text{SO}_4$ up to about 1.0 g. per liter, and was somewhat enhanced over the remainder of the range up to 10.0 g. per liter.

Influence of Cyanide.—Inasmuch as cyanide is known to inhibit the cytochrome system of respiration but not the riboflavin mechanism, it seemed desirable to test the effect of cyanide upon the formation of riboflavin by the yeast. The effects of cyanide upon growth and riboflavin production were studied in two experiments. Cyanide stock solution was prepared by dissolving KCN in distilled water. This solution was sterilized by filtration through a Berkfeld filter and small measured volumes were then



FIGURES 1-4

Growth and production of riboflavin by a yeast in relation to chemical compounds present in the nutrient medium. (1) Sugar is required for growth and synthesis of riboflavin, and amounts between 20 and 50 g. per liter appear to be near the optimum. (2) Potassium phosphate supplied from 0.5 to 1.0 g. per liter is near optimum; further increase of the salt stimulates growth somewhat but decreases the production of riboflavin. (3) With an increasing supply of ammonium sulphate as the sole source of nitrogen, both growth and synthesis of riboflavin are augmented. (4) Addition of small amounts of cyanide to growing cultures stimulates the synthesis of riboflavin but inhibits growth of the yeast.

added to the autoclaved and cooled basal culture medium to produce concentrations of KCN of 10^{-3} and 10^{-4} molar. Following inoculation of the cyanide treated media, growth and riboflavin formation were inhibited for a

period of several days, but after about a week the yeast began to grow and produce good yields of riboflavin.

In another experimental series kept at 25°C. potassium cyanide was added aseptically in different amounts to 50-ml. cultures which had been growing for about 24 hours. The production of the vitamin was increased appreciably by the presence of cyanide in the range of 1 to 4×10^{-4} molar, as indicated in figure 4. Growth of the yeast, however, was hindered by cyanide at all dosage levels. The addition of small amounts of cyanide to growing cultures would seem to offer a method for speeding the production of vitamin B₂. Although the mechanism by which cyanide increases the formation of riboflavin is not known, the phenomenon is of considerable interest in view of the well-known action of cyanide in poisoning the cytochrome system in aerobic organisms.

Effect of Aeration and Temperature.—In order to determine the influence of oxygen upon growth and yield of riboflavin, cultures were set up at 27°C. in air and in nitrogen contained in an anaerobic jar. At the end of six days, growth of the yeast was about the same in the two situations, but the production of riboflavin was reduced to a very low level in nitrogen. In another experiment performed with filtered air bubbling continuously through the medium, the yield of riboflavin was diminished as compared with the amounts formed by cultures kept in still air. When 50 ml. of medium were employed instead of 25 ml. in a 125-ml. flask, the production of riboflavin was diminished, perhaps because of suboptimum supply of oxygen.

For the purpose of ascertaining the effect of temperature upon growth and yield of riboflavin, a series of 50-ml. cultures were allowed to develop at 20°, 27°, 30° and 36°C. for one week. Multiplication of the yeast was rapid in the range 27° to 30°C., and the yield of the vitamin greatest at 30°C.

Summary.—Certain strains grown for a period of four to six days in synthetic culture media have been found to produce from 10 to 60 micrograms of riboflavin (vitamin B₂) per ml. of fermented liquor, depending upon environmental conditions. High yields of the vitamin are obtained by supplying certain organic nitrogenous compounds such as asparagine, glycine and methionine in the medium. The addition of hydrolyzed casein permits excellent growth, but inhibits formation of the vitamin. Sucrose, dextrose or levulose serve as suitable sources of carbon for growth and production of riboflavin. Synthesis of the vitamin is inhibited by the presence of maltose or galactose, though growth is not diminished by these sugars. Appropriate concentrations of salts, a temperature of about 30°C. and suitable aeration are important for obtaining efficient yields of the vitamin. Under anaerobic conditions the formation of riboflavin is inhibited without any decrease in growth. Small amounts of cyanide added aseptically to growing cul-

tures stimulated production of the vitamin but inhibited growth. It is apparent that synthesis of protoplasm and the production of riboflavin are related in different ways to the utilization of various compounds present in the culture medium. The ability of this yeast to synthesize extraordinary amounts of riboflavin appears to be genetically determined, but the extent to which this synthetic capacity is expressed depends a great deal upon cultural conditions.

* For obvious reasons it seems best not to publish the specific name of this yeast at present.

PSEUDOPYRIDOXINE AND CERTAIN FUNGI

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Snell, Guirard and Williams,¹ Snell² and Snell and Guirard³ have reported a series of observations on the relation of *Streptococcus lactis* R to pyridoxine from which the following conclusions, among others, may be drawn.

1. Pyridoxine as such is inactive.
2. An active compound, pseudopyridoxine, is formed when pyridoxine is autoclaved with amino acids.
3. Pyridoxine may be completely replaced in its growth effect by sufficient amounts of *dl*-alanine.

We have been interested in determining whether these conclusions apply to certain filamentous fungi which are known to be pyridoxine-deficient.⁴

The organisms used were *Ophiostoma catonianum*, *Ceratostomella ips* No. 255, *C. microspora*, *C. montium*, *C. multiannulata*, *C. piliferum*, *C. pluriannulata* and *C. ulmi*. Each fungus was grown in duplicate at 20°C. in 25 ml. of a basal mineral-dextrose medium containing asparagine and supplemented with biotin and thiamine.⁵ Observations on growth were made at intervals and after from 9 to 21 days, depending upon the rapidity of growth of the fungus, dry weights were determined by filtering into Gooch crucibles and drying at 100°C.

Activity of Pyridoxine.—In our experiments pyridoxine as such was effective for all eight of the fungi investigated. This was demonstrated by the following observations:

Little or no growth occurred in the basal solution. The addition of pyridoxine permitted considerable growth and no consistent significant

differences were noted in the action of pyridoxine sterilized with the constituents of the basal solution or added after the basal solution had been previously autoclaved (table 1). In our basal solution asparagine serves as a source of nitrogen. In order to eliminate the possibility that asparagine and pyridoxine might yield an active substance the asparagine was replaced by an equivalent amount of nitrogen in the form of NH_4NO_3 . Again little or no growth was observed without the addition of pyridoxine to the medium, and the activity of the pyridoxine was not increased by sterilizing it with the nutrient solution as compared to adding it after the basal medium had been autoclaved (table 1).

TABLE 1

DRY WEIGHTS IN MG. PER CULTURE OF FOUR FUNGI IN A BASAL SOLUTION SUPPLEMENTED WITH PYRIDOXINE, CASEIN HYDROLYSATE AND L-CYSTINE HCl

ADDITIONS TO 25 ML. OF BASAL SOLUTION	<i>Ophiostoma</i> <i>calonianum</i>		<i>C. pluriannulata</i>		<i>C. montium</i>		<i>C. microspora</i>	
	PYRI- DOXINE ADDED AFTER AUTO- CLAVING		PYRI- DOXINE ADDED AFTER AUTO- CLAVING		PYRI- DOXINE ADDED AFTER AUTO- CLAVING		PYRI- DOXINE ADDED AFTER AUTO- CLAVING	
1 m μ mole pyri- doxine	25.2	23.3	22.2	28.8	12.4	14.8	8.9	9.1
20 mg. casein hy- drolysate, 0.5 mg. l-cystine, 1 m μ mole pyridoxine	27.4	27.1	34.8	48.5	3.8	4.9	9.5	9.1
20 mg. casein, hy- drolysate, 0.5 mg. l-cystine	0.8	..	1.0	..	0.1	..	0.2	..
Asparagine re- placed by 0.5 mg. NH_4NO_3 , 1 m μ mole pyridoxine	18.6	19.2	19.8	21.6	10.9	11.3	5.9	8.4
None	0.3	..	0.2	..	0.1	..	0.1	..

Evidence for Pseudopyridoxine.—No evidence was obtained for the existence of a more active form of pyridoxine. Growth in the basal medium plus 1, 5 or 10 mg. of *dl*-alanine, and 1 m μ mole of pyridoxine per flask was approximately the same whether the alanine was sterilized with the pyridoxine in the nutrient solution or added under sterile conditions after the balance of the solution had been autoclaved (table 2). Growth in the basal medium plus 20 mg. of casein hydrolysate,⁶ 0.5 mg. of l-cystine HCl and 1 m μ mole of pyridoxine per flask was the same whether the pyridoxine was sterilized along with the other constituents of the nutrient solution or added after the balance of the solution had been autoclaved (table 1).

We expected that the response of the fungi might be greater in the medium containing pseudopyridoxine, i.e., the one in which the amino acids and pyridoxine were autoclaved together. No evidence for this effect was obtained.

Substitution of dl-Alanine for Pyridoxine.—We were unsuccessful in attempts to substitute *dl*-alanine for pyridoxine. Growth was not improved by the addition to the basal solution of 1, 5 or 10 mg. of alanine per flask. The same result was obtained when the alanine was autoclaved in the nutrient solution, and when it was added after the balance of the solution had been autoclaved (table 2). Since growth was obtained when pyridoxine was added to the solutions containing alanine, it appears that the lack of growth in the presence of alanine and absence of pyridoxine was not because of a toxicity of alanine, but because that substance did not replace pyridoxine for these fungi.

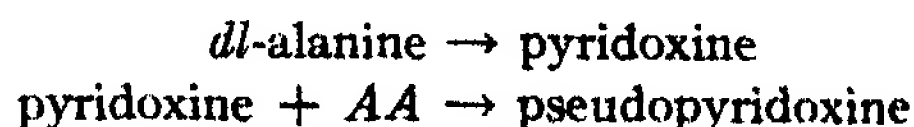
TABLE 2

DRY WEIGHTS IN MG. PER CULTURE OF FOUR FUNGI IN A BASAL SOLUTION SUPPLEMENTED WITH *dl*-ALANINE AND PYRIDOXINE

ADDITIONS TO 25 ML. OF BASAL SOLUTION	<i>Ophiostoma calonianum</i>		<i>C. pluriannulata</i>		<i>C. montium</i>		<i>C. microspora</i>	
	ALANINE ADDED AFTER AUTO- CLAVING	ALANINE ADDED AFTER AUTO- CLAVING	ALANINE ADDED AFTER AUTO- CLAVING	ALANINE ADDED AFTER AUTO- CLAVING	ALANINE ADDED AFTER AUTO- CLAVING	ALANINE ADDED AFTER AUTO- CLAVING	ALANINE ADDED AFTER AUTO- CLAVING	ALANINE ADDED AFTER AUTO- CLAVING
10 mg. alanine	0.5	0.2	0.3	0.6	0.5	0.4	0.8	0.3
5 mg. alanine	0.6	0.5	0.3	0.2	0.4	0.7	0.7	0.2
1 mg. alanine	0.4	0.1	0.2	0.3	0.5	0.4	0.7	0.4
10 mg. alanine, 1 mμ mole pyridoxine	29.2	31.3	26.3	29.6	25.7	21.6	14.4	14.4
5 mg. alanine, 1 mμ mole pyridoxine	29.9	26.5	32.3	24.6	25.3	24.0	12.9	14.1
1 mg. alanine, 1 mμ mole pyridoxine	31.9	26.8	36.1	28.0	24.5	22.7	13.6	13.5
1 mμ mole, pyridoxine	31.5	..	25.6	..	20.9	..	15.1	..
None	0.4	..	0.3	..	0.4	..	0.3	..

Discussion.—From our experiments it appears that the eight fungi we used differ from *S. lactis* R in their response to pyridoxine and to *dl*-alanine. Snell and Guirard⁸ state that for *Lactobacillus casei* also alanine does not replace pyridoxine, but that *S. lactis* 125 responds as does *S. lactis* R.

If we assume in accordance with Snell and his associates that



and that pseudopyridoxine is the active substance, then the following conclusions and assumptions would follow:

The eight fungi we investigated are unable to transform *dl*-alanine to pyridoxine under the conditions of our experiments.

These fungi produce adequate amounts of *AA* from sugar and inorganic nitrogen, but are unable to synthesize pyridoxine. When furnished pyridoxine they synthesize *AA* and combine the two intermediates (pyridoxine and *AA*) into pseudopyridoxine. This situation would be analogous to that which exists in the relation of the fungus *Ceratostomella montium* and numerous others to thiamine and its intermediates. *C. montium* is able to synthesize the thiazole portion of the thiamine molecule from the elementary constituents of a basal medium, but cannot make the pyrimidine intermediate. Furnished pyrimidine this fungus constructs thiazole and combines the two substances into the essential thiamine.⁶

On the basis of the assumptions made above we might expect to find organisms with the following deficiencies for pseudopyridoxine and its immediate precursors.⁷

1. Those requiring for growth the presence in the medium of pseudopyridoxine as such. This group would include organisms unable to synthesize pseudopyridoxine from its intermediates. *S. lactis* R and *S. lactis* 125 would probably belong in this group.

2. Organisms able to grow if supplied with both pyridoxine and the *AA* factor, but unable to grow with either one alone. These would be unable to construct either constituent of pseudopyridoxine, but would be capable of making the active pseudopyridoxine if furnished its intermediates. Animals would belong to this group or the next.

3. Organisms able to grow with pyridoxine present in the medium. This group would be able to synthesize *AA* but not pyridoxine. Supplied with pyridoxine they could construct *AA* and combine the two intermediates to form pseudopyridoxine. The eight filamentous fungi we used, and perhaps *Lactobacillus casei* also, would belong to this group.

4. Organisms able to grow with *AA* but no pyridoxine in the medium. This group would include those able to synthesize pyridoxine but unable to make the *AA* factor.

The validity of the suggested classification of organisms in relation to pseudopyridoxine depends, of course, upon further information on the existence, origin and composition of pseudopyridoxine and upon additional observations on its significance for various organisms.

Summary.—Eight filamentous fungi were found to respond to pyridoxine as such. The physiological activity of pyridoxine was not replaced by *dl*-alanine. No evidence was obtained from these fungi for the existence of a more active form of pyridoxine, Snell's pseudopyridoxine.

¹ Snell, E. E., Guirard, B. M., and Williams, R. J., *Jour. Biol. Chem.*, **143**, 519-530 (1942).

² Snell, E. E., *Proc. Soc. Exp. Biol. Med.*, **51**, 356-358 (1942).

³ Snell, E. E., and Guirard, B. M., *Proc. Nat. Acad. Sci.*, **29**, 66-73 (1943).

⁴ Robbins, W. J., and Ma, R., *Bull. Torrey Club*, **69**, 184-203 (1942). Robbins, W. J., and Ma, R., *Arch. Biochem.*, **1**, 219-229 (1942). Robbins, W. J., and Ma, R., *Am. Jour. Bot.*, **29**, 835-843 (1942).

⁵ The basal solution contained per liter 50 g. dextrose, 1.5 g. KH_2PO_4 , 0.5 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 2 g. asparagine. To this solution the following trace elements were added in p. p. m.: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo and 0.09 Zn. To each flask there were added also 0.1 μg . biotin methyl ester and 10 m μ moles thiamine. For the experiment summarized in part in table 1 the reaction of the basal solution was adjusted with KOH to pH 5.2 and for the one presented in table 2 to pH 5.8. Solutions were autoclaved at 15 lbs. pressure for 30 minutes.

⁶ We used S. M. A. vitamin-free casein hydrolysate which had been treated with excess CaCO_3 to neutralize the acidity.

⁷ Robbins, W. J., and Ma, R., *Bull. Torrey Club*, **70**, 190-197 (1943).

THE DISTRIBUTION OF PHOSPHATASE IN THE SPINAL CORD OF CHICK EMBRYOS OF ONE TO EIGHT DAYS' INCUBATION*

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Despite the enormous interest that has grown around the phosphatases since their discovery 36 years ago, our knowledge of the rôles they play in the economy of living organisms, in which they are almost ubiquitous, is but slight. In part the reason for this lack is, as Kay (1932) pointed out in another connection, the fact that the method of studying phosphatase activity in tissue extracts precludes any chance of discovering the intimate distribution of the enzyme within the tissues. In 1939, however, Gomori, and also Takamatsu, offered a histochemical method whereby the localization of alkaline phosphatase in cells and tissues may be determined with great exactness, and more recently Gomori (1941*b*) has extended this method to acid phosphatase.

With the thought that a histochemical study of the embryogenesis of these enzymes might produce clues concerning their biological significance, the writer undertook a comprehensive survey of the development and distribution of phosphatases in the embryo of the chick. Among the striking results to emerge from this study is the fact that both these enzymes appear in a remarkable pattern in the embryonic spinal cord.

Method.—The techniques outlined by Gomori (1941*a*, *b*) for the histological demonstration of alkaline and acid phosphatases were followed.

They were used on White Leghorn embryos of one to eight days' incubation, which were fixed in ice-cold acetone for 24 hours, embedded in a paraffin-beeswax-bayberry wax mixture by routine methods, and sectioned serially at 15 micra. Chilled acetone proved an excellent fixative for the gross histological examination of objects even as large as eight-day chick embryos, but the cell structures were somewhat distorted by shrinkage.

The sodium glycerophosphate used contained equal parts of the alpha and beta salts; pure alpha glycerophosphate was not available. The incubating solution for alkaline phosphatase was made according to Gomori's (1941a) instructions, at pH 9.3. Incubation was continued for three hours at 38 degrees. After treatment with cobalt nitrate and ammonium sulfide, the sections were counter-stained lightly with erythrosin. To compensate for the precipitation of lead beta-glycerophosphate, the formula (Gomori, 1941b) for the incubating solution for acid phosphatase, was altered as follows:

Molar acetate buffer at pH 4.8.....	2 ¹ / ₂ parts
5% PbNO ₃	2 parts
Distilled water.....	30 parts
Na glycerophosphate.....	5 parts

The solution was made up fresh for each test, and the precipitate removed by filtration. The pH is 5.1. Incubation was continued for six hours at 38 degrees. The sections were then treated with sulfide and counter-stained as before. Collodion protection was not found useful in either case.

The alkaline phosphatase showed remarkable stability in the course of handling. Keeping the specimens in acetone in the refrigerator for a week did not cause any variation in the excellently uniform pictures, nor did allowing the paraffin blocks or even the unstained mounted sections to stand for a month have any deleterious effect. The acid phosphatase, on the other hand, proved extremely labile, even on standing at icebox temperature, so that the achievement of comparable results required that the embryos be processed as rapidly as possible; three to four days were generally allowed from killing to staining. The embedding heat also had to be controlled carefully for, unlike the enzyme reported by Gomori (1941b) for adult mammalian tissues, this phosphatase was destroyed by temperatures above 60 degrees. Yet, in spite of these precautions, individual embryos showed considerable variation in the intensity of acid phosphatase activity. The pattern of distribution in the various tissues, however, was constant.

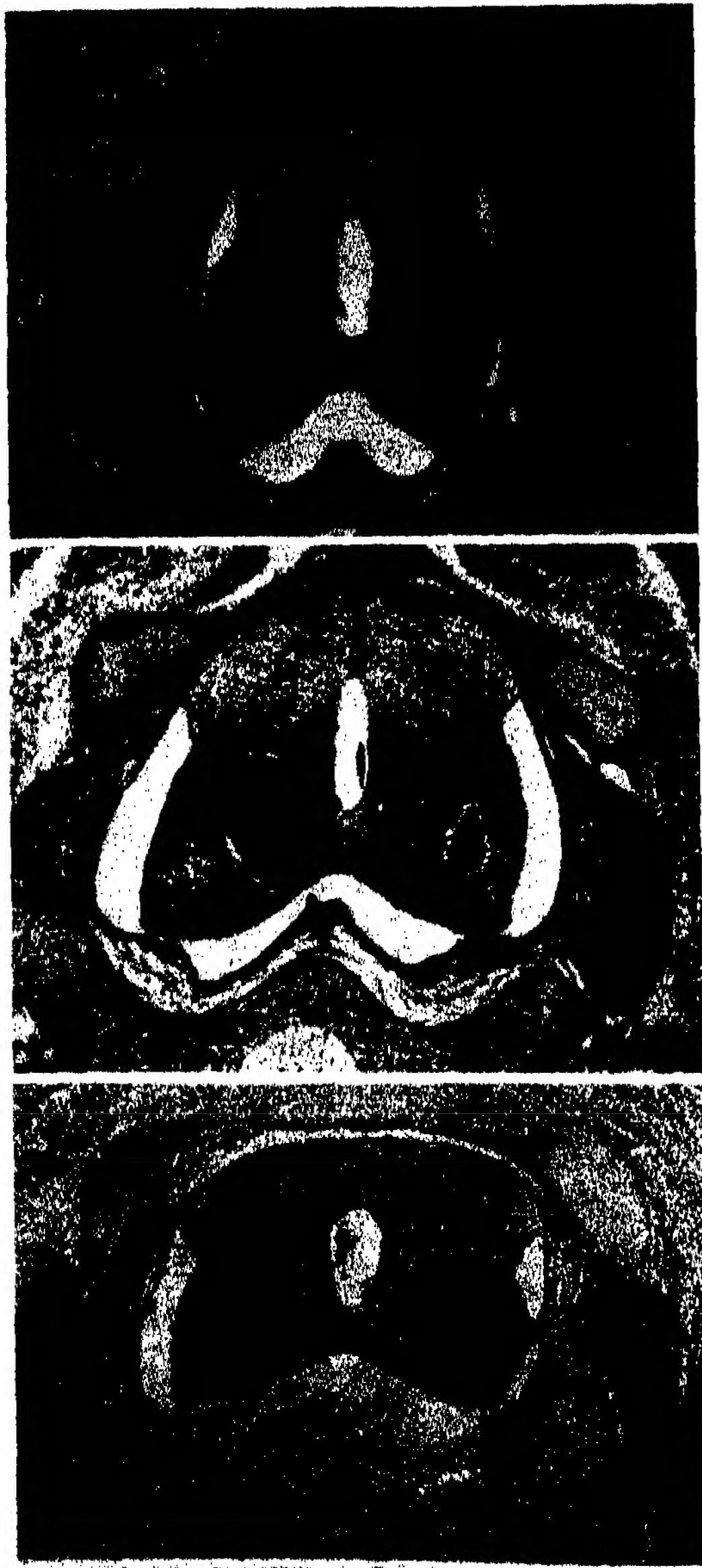
Results.—(1) *Alkaline Phosphatase.* The activity of alkaline phosphatase, as judged by the amount of phosphate deposited within a given time,

is generally strong in the embryo, for heavy black deposits appear in many tissues even if incubation is continued for only one hour. A high concentration of alkaline phosphatase is found in the neural tissue at the end of the first day, the earliest stage examined. Through the second day the neural tube is uniformly active at anterior levels, but near the end of the region of differentiated somites the reactivity wanes and almost disappears. Anteriorly, on the third day, the dorsal half of the cord and the neural crests tend to react more strongly than the ventral half, but this difference fades into uniformity at posterior levels. On the next two days small portions of the ventral region of the cord continue to lose their alkaline phosphatase content, and it soon becomes clear that these portions represent the future motor horns of the limb plexus. The white matter differentiating outside of the horn area is strongly positive. In the tail region, on the fourth day, the distribution is still fairly uniform.

From the end of the fourth day on, the enzyme disappears according to an antero-posterior gradient from the dorsal half of the gray matter, while it becomes more concentrated in the ventral half, so that by the seventh day a definite and apparently persistent pattern is established. The striking feature of this pattern is a heavy black crossband that virtually bisects the cord; the band reaches from the lumen to the outer edge, thus involving ependymal, marginal and mantle layers. Dorsal to the band the ependyma and gray matter are almost negative, except for a small positive area just above the lumen, at the base of the dorsal funiculus; but ventrally both layers show a marked reaction, with the exception of a limited portion of the ependyma immediately under the lumen, and of the large lateral motor groups which, by the sixth day, are established in the pelvic as well as the pectoral region. The mesial motor groups are also less active than the surrounding gray matter, but the demarcation is not as sharp as in the other cases cited. The white matter contains a high concentration of alkaline phosphatase throughout, though the activity is greater ventral to the crossband than dorsal to it; the ventral commissure is extremely active.

This pattern does not change through the eighth day. Its appearance in the cervical and fore-limb regions is shown in figures 1 and 2.

The absence of alkaline phosphatase described refers only to the cytoplasm of the cell bodies and processes in question. Within such cells the nuclear membranes are clearly outlined by black deposits, and the nucleoli are also stained black. In the cells which show phosphatase in their cytoplasm, when the deposits are light enough to permit examination of the interior of the cell, it can be seen that the nuclear membranes and nucleoli are also strongly reactive. There are no cytoplasmic inclusions seen in either case.



FIGURES 1-3

All sections are from one eight-day embryo. Magnification 50 \times . (1) Alkaline phosphatase, neck region. The heavily stained distal portions of the ganglia are made up of the large cells. (2) Alkaline phosphatase, fore-limb region. (3) Acid phosphatase, hind-limb region. Note the darkly stained masses in the large cells of the ganglia.

In the sensory ganglia, which are made up of cells of two sizes, a clear-cut disparity exists in the alkaline phosphatase content of the cytoplasm of the large and the small cells: the former reacts strongly, whereas the latter seems devoid of the enzyme. Thus the clustering of the large cells distal to the nerve cord gives the phosphatase-stained ganglion the appearance of being divided into two parts (see Fig. 1). As in the cells of the cord, the nucleoli and nuclear membranes of the small cells stain deeply, and in addition a faint darkened network, probably of chromatin, may occasionally be seen inside the nucleus. The interior of the large cells is obscured by the heavy sulfide deposits.

The dorsal and ventral roots are strongly positive, as are the nerve trunks peripheral to the ganglia.

Tests run on six-day embryos at pH 8.5, 8.0 and 7.5 showed that the activity is markedly less at pH 8.5 than at 9.3, and it is abolished at pH 8.0 or lower. Other tests, run for one hour, in which the incubating solution was made 0.001 *M* and 0.01 *M* with respect to $MgCl_2$, revealed some activation at the lower concentration and very marked activation at the higher.

(2) *Acid Phosphatase*. Like its alkaline counterpart, acid phosphatase also appears in the neural tissue at the end of the first day, but it does not seem to be as concentrated, or as active, as the alkaline enzyme. Slides treated by the alkaline technique regularly show dense black deposits, whereas after the acid treatment the deposits in the nerve tissue never have more than a deep golden brown color, even when the incubation is continued for 12 hours. Strict comparison between the two enzymes on this basis is not possible, however, since the acid phosphatase could operate on alpha-glycerophosphate only; and the beta salt is the preferred substrate of the phosphomonoesterases of both Class AI and Class AII (Folley and Kay, 1936). But it is noteworthy that Fleischhacker (1938) found a similarly great disparity between the activity of the two enzymes in mammalian brain tissue, even when beta-glycerophosphate was the substrate for both.

As the cell layers of the cord differentiate, acid phosphatase becomes concentrated chiefly in the ependyma, while the surrounding tissue shows lessened activity. Between the fifth and the seventh day acid phosphatase largely disappears from all three layers of the dorsal half of the cord, except for a few cells just dorsal to the lumen—the same spot that also shows alkaline phosphatase activity. In the ventral half the enzyme occurs in reactive cells scattered through a negative ground substance; but heavier uniform concentrations are found in the lateral and mesial motor groups. These deposits appear in posterior progression, demarcating the lateral horn in the fore-limb region on the fifth day, and in the hind-limb region less than a day later. The ventral commissure also stains more heavily

than its surroundings, and so do an extensive group of ependymal cells below the lumen.

Thus a definite pattern, as shown in figure 3, is laid down by the end of the sixth day. Aside from more complete withdrawal of the enzyme from the dorsal half of the cord, the pattern does not change essentially through the eighth day.

Unlike alkaline phosphatase, the acid enzyme is apparently absent from the nuclei of cells which have unreactive cytoplasm. In the reactive cells the nuclei stain more lightly than the cytoplasm and show no inclusions; but there is in each cell a small, intensely stained body outside the nucleus but closely applied to it. The nature of these bodies is not evident.

The small cells of the ganglia appear to contain only a little acid phosphatase in their nuclei and cytoplasm; they do, however, show the same reactive body on the surface of the nuclei as was found in the active cells of the cord. The large cells contain a very large, intensely reactive mass which is bigger than the nucleus and apparently includes all the cytoplasm of the cell body except for a negative rim continuous with the processes. The clump has a granulated appearance which seems to be caused by darkly staining inclusions. Possibly it represents the tigroid substance shrunk into a rounded lump (see Fig. 3).

The dorsal and ventral roots, and the nerve trunks leading from the ganglia, are lightly positive.

Discussion.—In a consideration of the nature of these enzymes, the thought arises that the alkaline phosphatase, at least, is a phosphomonoesterase component of a lecithinase, a substance which might reasonably be expected to occur in nerve tissue. Comparison of the pH tests reported here with those of King (1931), however, enable the dismissal of this possibility; for King showed that lecithinase has a pH optimum of 7.0–7.4, whereas the alkaline phosphatase of this study did not act at all at pH 8.0 or lower. Similarly it can be seen that neither the acid nor the alkaline phosphatase resembles the 5-nucleotidase found widespread in nerve tissue by Reis (1937). The Reis enzyme is only slightly active against glycerophosphate, but these enzymes are as effective on this substrate as are most other phosphatase-containing tissues of the embryo. Although the 5-nucleotidase is virtually inactive at pH 5, it retained considerable activity at pH 9, but again it fails to correspond to the alkaline enzyme of this study in being unaffected by the presence of magnesium ions.

The enzymes found in the embryonic chick cord do, however, correspond well with the brain phosphatases delineated by Fleischhacker (1938) and Kotkova (1939). The latter demonstrated in the brain of the sparrow and pigeon, as well as of members of other classes, phosphomonoesterases with pH optima of 4.5–5.6, and 8.9–9.6; the second was activated by magnesium, and the first inhibited by fluoride. Fleischhacker obtained somewhat

similar results, except that he claimed the acid phosphatase to be activated by magnesium; but the acid activities he reported were in all cases so low that the differences may be unreliable. Probably all these nerve phosphatases can be classified, according to Folley and Kay's (1936) scheme, as phosphomonoesterases of Classes AI and AII. They may be concerned in the metabolism of the carbohydrate substrates of nerve tissue.

The change in the distribution of the embryonic cord enzymes with time indicates that they may have more than one function. The uniform distribution of the enzymes in the neural tissue of the earliest stages, and their later disappearance from certain regions, and concentration in others, are phenomena shared by practically every organ of the embryo (unpublished results). Thus the observer is led to feel that the phosphatases are essential in the initiation, at least, of differentiation; for every indifferent cell contains phosphatase in its cytoplasm. As differentiation proceeds, the enzymes may disappear, or they may become more concentrated. In the latter case the accumulation of the enzymes in local regions may be regarded as a consequence of differentiation in itself, or in other words a chemical differentiation paralleling the histological differentiation; presumably the enzymes are thereafter concerned in the functioning of the specific cells in which they are localized. In this connection it is suggestive that the establishment of the pattern described, and especially the accumulation of acid phosphatase in the motor groups, precedes by about 24 hours the appearance (at 6½ to 7 days) of the first local reflexes (Orr and Windle, 1934). In any case the results suggest for phosphatase a hitherto unsuspected rôle in the actual functioning of nerve elements.

That the localization of acid phosphatase at least is not a transitory embryonic phenomenon is shown by the fact that Fleischhacker (1938), using a crude histochemical method adapted directly from the colorimetric method of Kuttner and Lichtenstein, found similar concentrations in the anterior horn cells of adult rats; he did not mention the pH. Gomori's (1941a) failure to find any alkaline phosphatase in mammalian nerve tissue, however, remains to be explained.

Summary.—1. The presence of acid and alkaline phosphatases in the spinal cord of the embryonic chick has been demonstrated by the histochemical techniques developed by Gomori.

2. At all stages examined, alkaline phosphatase reacts much more strongly than acid phosphatase.

3. As early as the end of the first day of incubation, both acid and alkaline phosphatase are found in fairly high concentration in the neural tissue.

4. As development proceeds, the alkaline phosphatase becomes localized in the white matter throughout, and in the gray matter and ependyma of the ventral half of the cord, with the exception of the cells of the motor

groups. It is absent from a small part of the ependyma just below the central canal, and is concentrated in a similar small area just above the canal.

5. The acid phosphatase becomes largely restricted to the ventral half of the cord, and is especially concentrated in the motor groups. It also occurs in small regions just dorsal and ventral to the lumen.

6. The pattern described appears in antero-posterior progression on the fourth, fifth and sixth days.

7. The large cells of the spinal ganglia contain large amounts of both acid and alkaline phosphatase, the small cells very little of either.

8. As far as can be detected, all nucleoli and nuclear membranes are positive for alkaline phosphatase.

9. Acid phosphatase is not found in nuclei, but even where the cytoplasm is not reactive the enzyme is generally present in a small body, of unidentified nature, which is closely applied to the nucleus. In the large ganglionic cells, the cytoplasm is not uniformly reactive, but contains a very large rounded clump which is strongly positive.

10. Evidence is presented which indicates that these enzymes are phosphomonoesterases of Classes AI and AII.

11. It is suggested that both these phosphatases first play a part in early differentiation, and later are involved in the specific functions of the cells in which they become concentrated.

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STUDIES ON THE METHANE FERMENTATION. VI. THE INFLUENCE OF CARBON DIOXIDE CONCENTRATION ON THE RATE OF CARBON DIOXIDE REDUCTION BY MOLECULAR HYDROGEN

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The methane-producing bacterium *Methanobacterium omelianskii* has been shown^{2, 3, 4} to form methane by reduction of carbon dioxide, using primary and secondary alcohols and molecular hydrogen* as hydrogen donors. As an extension of earlier studies on this process, it seemed desirable to find out how the concentration of carbon dioxide influences the rate of its reduction. In studying this effect, the reaction



has been used because the large gas volume change is advantageous for making rate measurements by a manometric technique.

Methods.—A pure culture of *Mb. omelianskii* was grown in a previously described² ethanol-bicarbonate medium which was incubated until 3.0 to 5.5 ml. *N*/10 acetic acid had been formed per 10 cc. The cells from a 500-ml. culture were separated by centrifugation, washed once, and resuspended in 10–15 ml. of a solution containing 0.2 per cent NaCl, 0.2 per cent KCl and 0.03 per cent Na₂S·9H₂O, pH 7. These operations must be carried out so as to minimize exposure of the cells to oxygen. The mixture of chlorides is preferable to distilled water because it increases the rate of sedimentation. The final suspensions contained 0.4–0.5 mg. cell-nitrogen per ml. and took up 5–7 × 10³ cmm. H₂/hr./mg. cell-N at high carbon dioxide tensions. If not used immediately the cells were stored at 3°C. *in vacuo*; under these conditions they retain their full activity for at least two days.

All rate measurements were made at 37°C. by the Warburg manometric technique.

The H₂-CO₂ Reaction.—Since our strain of *Mb. omelianskii* has been shown to form methane from hydrogen and carbon dioxide,⁶ it is only necessary to present indirect evidence that the same reaction occurs under the conditions of our manometric experiments. For this purpose the ratio H₂ uptake/CO₂ uptake, which according to equation (1) should equal 4, has been determined. A value of 3.53 was found (table 1). In arriving at this value, the hydrogen uptake was calculated from pressure changes on the assumption that one volume of methane was produced for each four volumes of hydrogen disappearing. Carbon dioxide uptake was calculated on the basis of the initial and final bicarbonate and the partition of carbon

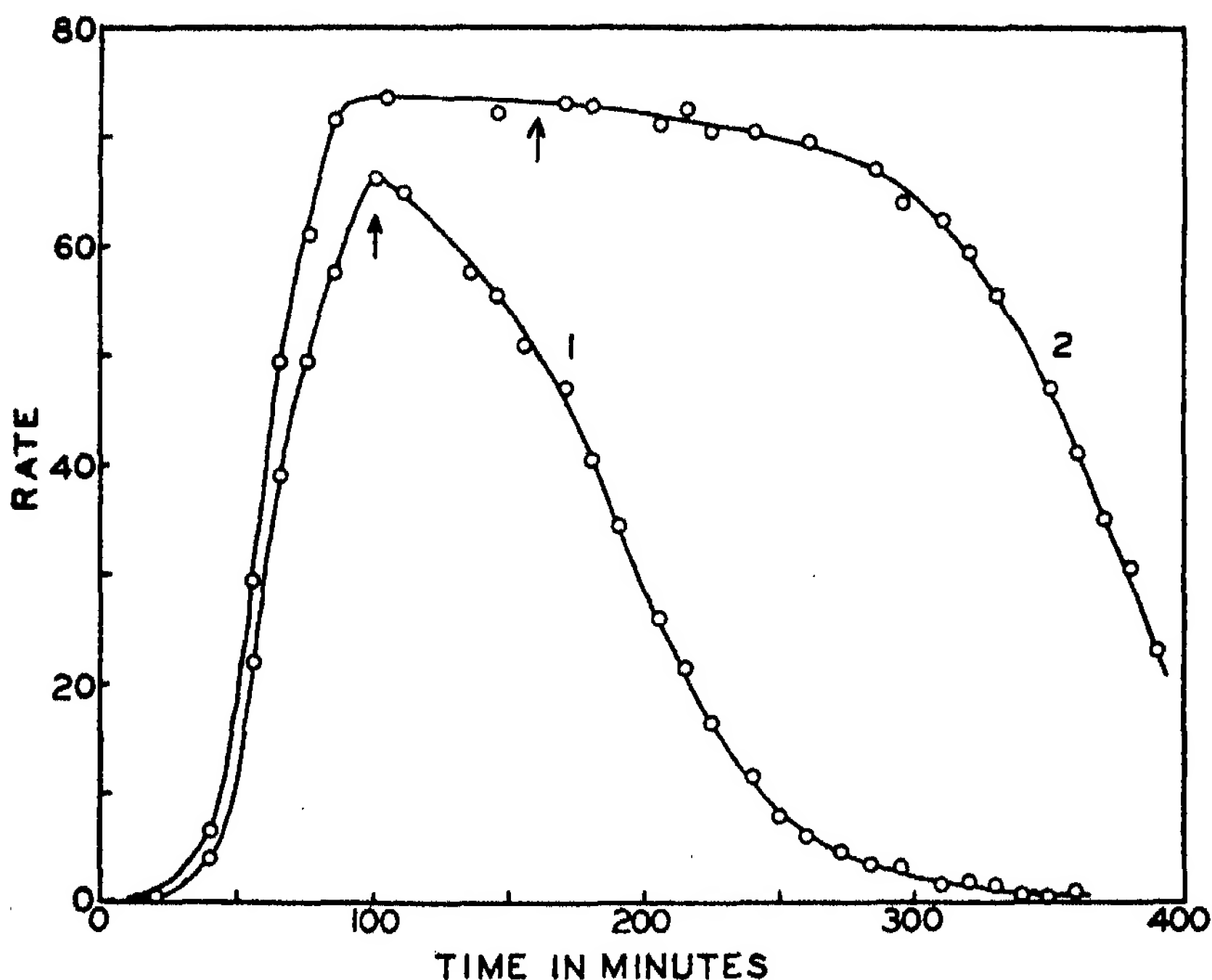


FIGURE 1

Experimental rate-time curves from which the rate-carbon dioxide concentration curve of figure 2 is derived. Each vessel contained 0.2 ml. of a bacterial suspension containing 0.069 mg. cell-N, 0.4-ml. *M*/2 phosphate buffer, pH 6.64, 0.3 ml. *M*/25 (Curve 1) or *M*/10 (Curve 2) bicarbonate-carbonate solution. Total volume, 2 ml. Gas phase, oxygen-free hydrogen, 37°C. The rate is expressed in arbitrary units.

dioxide between the liquid and gas phases. In control experiments the carbon dioxide production in the absence of hydrogen (N_2 atmosphere) was negligible and the rate of hydrogen uptake by cells suspended in a

TABLE 1

THE H_2 - CO_2 REACTION

	mm.
Initial CO_2	166.3
Final CO_2	73.9
CO_2 uptake.....	92.4
H_2 uptake.....	326.
H_2/CO_2	3.53

phosphate buffer was less than 10 per cent of that by cells in a bicarbonate buffer. In view of several approximations involved in calculating the hydrogen-carbon dioxide ratio by the above method, the agreement between the experimental value and equation (1) may be considered satisfactory.

Changes in Rate with Time.—When a cell suspension is allowed to act upon a mixture of hydrogen and carbon dioxide, a constant reaction rate is not immediately established. The rate is initially very low, but gradually increases until after a period varying from 30 minutes to as much as five hours, it reaches a maximum level which persists as long as an adequate supply of carbon dioxide and hydrogen is available. This phenomenon is illustrated in figure 1.

Since a long period of changing rate is obviously undesirable in manometric experiments, an attempt was made to find conditions under which the phenomenon could be eliminated. This attempt was not entirely successful; however, it was found that the maximum rate is reached much sooner with cell suspensions not more than one day old than with older cells. With old but not with young cells a two-hour pretreatment with hydrogen shortens the lag period considerably. The addition of 0.03 per cent $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ has no effect. In acid media ($\text{pH} < 6$) the lag period is greatly prolonged.

Influence of CO_2 Concentration.—There are two methods for studying the influence of carbon dioxide concentration upon the rate of a process in which carbon dioxide is used up. The multiple vessel method consists in comparing the initial reaction rates in a number of vessels to which different amounts of bicarbonate have been added. In the second, single vessel method, the decline in reaction rate is followed as carbon dioxide is used up. Preliminary experiments showed that the multiple vessel method cannot readily be applied with *Mb. omelianskii* because of the long lag period during which the initial carbon dioxide concentration is greatly reduced. If the initial concentration is small a constant rate is never attained. The single vessel method was therefore used for the final experiments. The well-known objection to this method, namely, that the accumulation of reaction products retards the rate, appears to be unimportant in the present instance.

In applying the single vessel method, a strongly buffered medium was used so the pH and consequently the ratio of carbonic acid to total carbonate would not change appreciably during the experiment. A pH 6.6 phosphate buffer was chosen in order that the rate would be nearly maximal and independent of pH (Fig. 3). The actual pH after addition of the bicarbonate-carbonate mixture was about 6.8. A hydrogen atmosphere was used. After temperature equilibration the bicarbonate was mixed with the cell suspension and the pressure decrease was followed for several hours until exhaustion of carbon dioxide reduced the rate practically to zero. The concentration of carbon dioxide at any given time was calculated from the initial and final levels by making the entirely justifiable assumption that the disappearance of carbon dioxide was proportional to the pressure change. In control experiments, the decline in bicarbonate was measured directly by adding excess acid after various intervals.

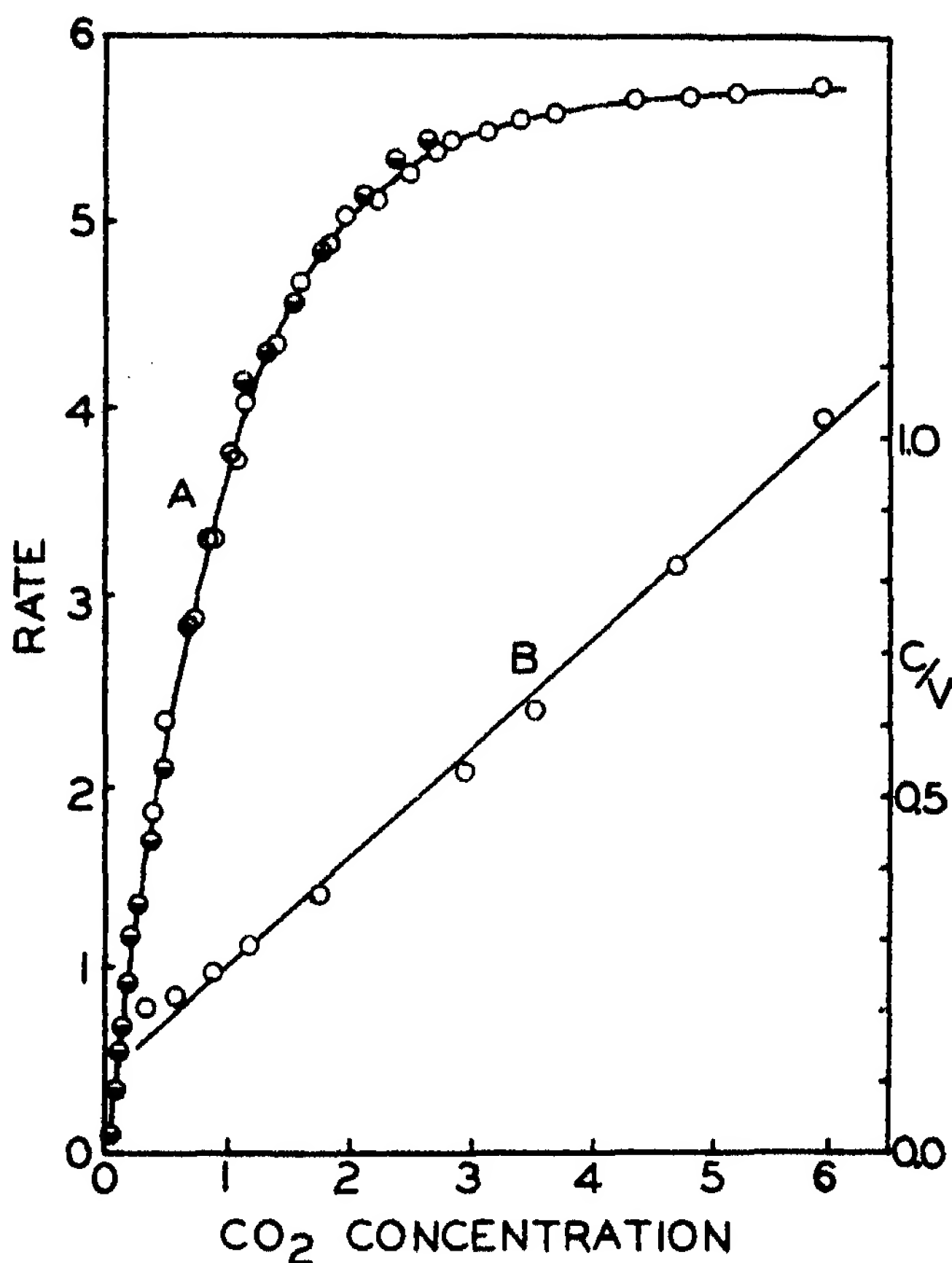


FIGURE 2

The influence of carbon dioxide concentration on the rate of hydrogen uptake (Curve A). The rate of hydrogen uptake is expressed in $\text{cmm. H}_2/\text{hr./mg. cell-N} \times 10^3$. Carbon dioxide concentration is expressed in $\text{moles/liter} \times 10^{-4}$. Curve B: The ordinate (right) is C/V , where C is carbon dioxide concentration and V is the rate of hydrogen uptake.

The primary data of a typical experiment are presented graphically in figure 1 and the derived carbon dioxide concentration-rate curve is given in figure 2, Curve A. In this experiment two vessels were used, one with 0.3 ml. $M/25$, the other with 0.3 ml. $M/10$ bicarbonate. With both vessels, about 100 minutes elapsed before a maximum reaction rate was reached. In vessel No. 1, with the smaller amount of bicarbonate, the rate immediately began to decline as a result of the decrease in carbon dioxide concentration. In vessel No. 2 with more bicarbonate, the rate re-

maintained almost constant for more than an hour following the maximum since carbon dioxide was not at first a limiting factor. The two vessels together cover most of the range in which carbon dioxide concentration controls the reaction rate. The points on Curve A, figure 2, are derived from the portions of the smooth curves of figure 1 to the right of the arrows.

Curve A of figure 2 shows that the rates of hydrogen uptake and carbon dioxide reduction change greatly with carbon dioxide concentration. The shape of the curve is similar to that for other enzymatic processes. The data fit the Michaelis-Menton equation only approximately as can be seen from Curve B (Fig. 2) in which C/V is plotted against C .⁷ The departure from linearity is greatest at low carbon dioxide concentrations where the experimental errors are largest.

The position of the rate-concentration curve on the concentration axis is of some interest. The half maximum rate is attained at a carbon dioxide concentration of about $7 \times 10^{-6} M$. With photosynthetic algae, the corresponding concentration is much lower, about $7 \times 10^{-6} M$ for *Chlorella pyrenoidosa*⁵ and $1 \times 10^{-6} M$ for the fresh water diatom *Nitzschia palea*.¹ Half saturation of the formic hydrogenlyase of *B. coli*⁸ occurs at a higher concentration, about $1.5 \times 10^{-3} M$. These comparisons indicate that bacteria are adapted to higher carbon dioxide tensions than algae. This difference can be correlated with normal differences in the carbon dioxide content of the environments in which the two groups of organisms live.

Influence of pH.—The above experiment does not allow one to decide whether the rate is determined by free carbon dioxide or by bicarbonate ion, since the concentrations of both substances vary simultaneously and proportionately. In order to decide which factor is the more important, the reaction rate was determined when the ratio of carbonic acid to bicarbonate was varied by changing the pH. Two types of pH-rate experiments were carried out. In one, the carbon dioxide concentration was kept constant by equilibrating the medium with a mixture of 8.75 per cent carbon dioxide in hydrogen. At this concentration ($2.2 \times 10^{-3} M$), carbon dioxide does not limit the rate. The pH was controlled by varying the bicarbonate concentration. The results of two experiments are given in table 2. In the second type of pH-rate experiment the total carbonate concentration was kept constant while the pH, carbon dioxide and bicarbonate ion concentrations were varied. The maximum rates of hydrogen uptake were determined in a medium containing 0.1 M phosphate or phosphate-acetate buffer and 0.010–0.015 M NaHCO_3 . The quantity of bicarbonate was large enough so that the carbon dioxide concentration would not decrease greatly in short experiments and was small enough to avoid any considerable change in pH of the buffer. The pH was determined with a glass electrode at the end of each experiment. The results of five experiments are summarized in figure 3.

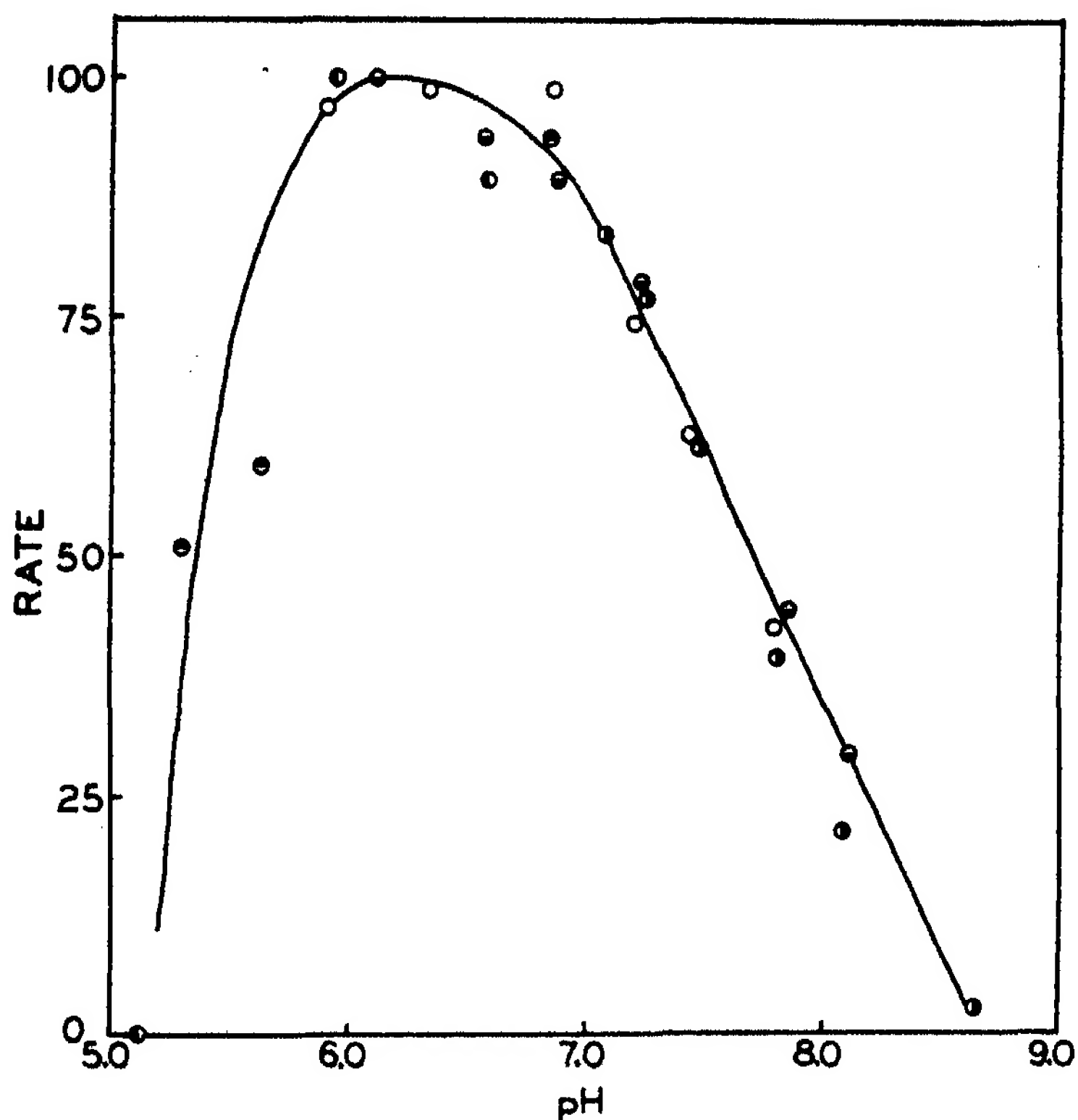


FIGURE 3

The influence of pH on the rate of hydrogen uptake, expressed in arbitrary units, when the total carbonate concentration is low (0.010-0.015 *M*) and constant.

TABLE 2

INFLUENCE OF pH ON THE RATE OF CARBON DIOXIDE REDUCTION AT A HIGH CO₂ TENSION (Gas phase: 8.75 per cent CO₂ in H₂)

APPROX. HCO ₃ ⁻ CONC.	pH	RELATIVE RATE
0.000	5.80	81
0.002	6.57	82
0.008	6.94	87
0.012	7.11	82
0.03	7.42	82
0.06	7.72	100
0.10	8.00	74
0.20	8.19	57

The data of table 2 show that when the partial pressure of carbon dioxide is kept constant, the rate is nearly independent of pH and bicarbonate concentration over the range from pH 5.8 to 7.4. At pH 7.7 the rate is

slightly greater while in more alkaline media it declines rapidly. On the basis of this experiment it is impossible to decide whether the decline is due to the alkalinity or to the high bicarbonate concentration.

Figure 3 shows that the rate declines with increasing pH over the range 6.4 to 8.6 in which carbon dioxide tension decreases and bicarbonate concentration increases. Since in this experiment the rate decreases with carbon dioxide tension, while in the previous experiment where the carbon dioxide tension was kept constant the rate was largely independent of both pH and bicarbonate concentration, one must conclude that carbon dioxide is the substance which combines with the catalytic system and primarily determines the rate at least over the pH range 6.0 to 7.8. A similar conclusion has been reached in the case of green plant photosynthesis.⁵ Above pH 7.8 and below pH 6 the rate is determined mainly by the concentrations of hydrogen or bicarbonate ions.

* The ability of the organism to use molecular hydrogen was discovered about 1940 by Charles Schnellen, working in the laboratory of Prof. A. J. Kluyver. Because of the war, Schnellen's thesis is not yet available.

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EFFECT OF COLCHICINE PRETREATMENT ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS INDUCED BY X-RADIATION

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Recent investigations indicate that the frequency of chromosomal aberrations induced by x-radiation is influenced by the amount of chromosomal movement during radiation. Increased chromosomal movement results in a higher frequency of aberrations since there is a greater opportunity for broken ends to fuse in new combinations. Thus centrifuging the cells during radiation tends to increase the frequency of induced aberrations (Sax¹). Prophase stages are more sensitive than resting stages partly because of greater movement in the prophasic nucleus. The differential

sensitivity of different types of cells and tissues to x-rays is apparently associated with differences in the amount of chromosomal movement at the time of radiation (Sax and Swanson²). Radiation at high intensity results in a greater aberration frequency than the same dose given at a low intensity, indicating that the frequency of illegitimate fusions depends on the time interval that the breaks remain "open" as well as the amount of movement during this interval (Sax³).

Colchicine inhibits anaphase movement of chromosomes and apparently retards other stages of the mitotic cycle (Eigsti⁴). Thus the use of colchicine offers another means whereby the relationship between the amount of chromosomal movement and the frequency of x-ray induced aberrations can be tested. The results presented here indicate that treatment with colchicine prior to radiation reduces the frequency of aberrations induced by x-radiation at the prophase stages.

Methods.—Onion bulbs of the White Marglobe variety were used. Four bulbs were germinated in water until a number of roots 2.0–2.5 cm. in length were developed. Two of them were placed with their roots dipping into a 0.05% solution of colchicine for 45 minutes, the other two serving as controls. The roots of the control bulbs and colchicine treated ones were then subjected to 300 r units of x-radiation, the x-ray treatment being given both lots simultaneously. Following radiation both lots were placed with their roots in a 0.05% solution of colchicine for 48 hours. At the end of this time the root tips were fixed in 3:1 alcohol-acetic, stained with the Feulgen stain and smeared.

Treatment with colchicine following radiation inhibits anaphase, thus insuring that none of the aberrations is lost by cell division subsequent to radiation and at the same time increases the number of metaphases available for study. Some tetraploid cells were found after this treatment, however, this report is limited to the effects observed in diploid cells of the pretreated roots and controls. A comparison of the types and frequencies of breaks in tetraploid and diploid cells will appear in a separate report (Sax and Brumfield⁵).

Experimental Results.—The types of aberrations observed in both the experimental and control roots were the same as those reported by Sax for x-irradiated *Allium* roots (Sax⁶) and *Tradescantia* microspores (Sax⁷). However, because of the use of the colchicine after radiation, secondary rings and dicentrics did not occur. The results are presented in table 1 which shows the frequencies, expressed as number of aberrations per 100 chromosomes, of different types of induced aberrations. As seen from the table, total aberration frequency in the controls was about twice that of the colchicine pretreated material. Chromosome effects were found with about the same frequency in both lots. Significant differences ($p < 0.01$) were found in the frequency of chromatid effects, both the one-hit and two-

hit aberrations being about 3.5 times as frequent in the controls as in the colchicine pretreated roots.

Discussion.—Chromatid effects are induced in cells irradiated during prophase stages when the chromosomes are effectively split while chromosome aberrations result from radiation of resting nuclei, the chromosome then being effectively single. Thus those cells having chromatid effects were evidently in prophase at the time of the colchicine pretreatment and x-radiation while those showing chromosome effects were in resting stages. The results presented here indicate that the colchicine pretreatment did not appreciably affect the frequency of aberrations induced in resting nuclei, but significantly reduced the frequency of aberrations induced in prophase stages. Chromosome movement is presumably at a minimum during the resting stage. Accordingly, colchicine pretreatment, by reducing chromosome movement, should have little differential effect on the frequency of chromosome aberrations induced in the pretreated roots as compared with the controls. During prophase, however, with the development of new minor coils and reduction in the number of relational coils, there is considerable movement of the chromosomes. This gives a greater opportunity for broken ends to fuse in new combinations and is partly responsible for the sensitivity of prophase nuclei being greater than that of resting nuclei. Slower movement would evidently result in reduced sensitivity to x-rays and this is most probably the mechanism whereby colchicine pretreatment affects a lower frequency of chromatid effects induced by x-radiation.

Guyer and Clark,⁸ found that cancer tissue pretreated with colchicine for 15 hours was more sensitive to x-radiation than untreated controls, and Levine⁹ reported that onion roots treated with a 0.01% colchicine solution for 48 hours were more sensitive than untreated roots. In these cases, however, the colchicine pretreatment was of sufficient duration to cause a considerable increase in the number of metaphases present, a stage of high sensitivity to x-radiation. Consequently these results are not directly comparable to the experiment presented here, since in this case, the 45-minute colchicine pretreatment was not sufficient to appreciably increase the number of metaphase stages (cf. Levine and Gelber¹⁰).

Summary.—Treatment of onion roots for 45 minutes with a 0.05% solution of colchicine prior to x-radiation resulted in less than one-third as many chromatid aberrations as found in non-colchicine treated controls. The frequency of x-ray induced chromosome effects was about the same in pretreated roots and controls. The colchicine pretreatment presumably reduces the amount of chromosome movement in prophase where chromatid effects are induced, thus resulting in less opportunity for fusion of broken ends into new combinations.

TABLE 1

SHOWING THE FREQUENCY OF ABERRATIONS, EXPRESSED AS NUMBER OF ABERRATIONS PER 100 CHROMOSOMES, INDUCED BY X-RADIATION IN ONION ROOTS TREATED WITH A 0.05% COLCHICINE SOLUTION FOR 45 MINUTES PRIOR TO X-RADIATION, AND CONTROLS.
300 R

	TOTAL CHROMO- SOMES	CHROMATID ABERRATIONS			CHROMOSOME ABERRATIONS			TOTAL ABERRA- TIONS
		ONE-HIT	TWO-HIT	TOTAL	ONE-HIT	TWO-HIT	TOTAL	
Colchicine								
pretreated	4448	0.79	0.24	1.03	0.94	0.81	1.75	2.78
Controls	6272	2.66	0.85	3.51	0.65	1.18	1.83	5.34

This experiment was carried out at Harvard University while the author was holder of a National Research Fellowship. The author wishes to express his sincere appreciation to Prof. Karl Sax for suggestions and criticisms.

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SOME TOPOLOGICALLY INVARIANT METRIC PROPERTIES

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According to the well-known duality theorem of Alexander, each simple closed curve C in cartesian n -space E_n is linked by an $n-2$ cycle Z in $E_n - C$. In the sequel it will be shown that Z can frequently be taken to be a metric $n-2$ dimensional sphere. A generalization will be formulated which holds not only in E_n , but in an arbitrary metric space. As an application, solutions will be given to two hitherto unsolved problems proposed, respectively, by L. M. Blumenthal and I. J. Schoenberg.

Let p and q be two points of a metric space M ; $D(p, q)$ the set of points x such that $px = xq \leq pq$; $S(p, q)$ the set of points x such that $px = xq = pq$; $H(p, q)$ the set of points x such that $px = xq$. $D(p, q)$, $S(p, q)$ and

$H(p, q)$ will be called, respectively, the disc, the sphere, and the subspace dual to p and q in M . $H(p, q)$ has two sides; the p -side consisting of the points of M whose distances from p are less than their distances from q , and a q -side similarly defined.

An arc A in M will be said to be in general position relative to a disc $D(p, q)$ if neither end-point of A is in $D(p, q)$ and A does not intersect $S(p, q)$. If A is in general position relative to a disc $D(p, q)$, then, in close analogy to the procedure for cartesian E_n , it is possible to define an intersection number $I(A; p, q)$ between A and $D(p, q)$. Taken mod 2, $I(A; p, q)$ will be either 0 or 1. For any arc A' sufficiently close to A , and points p', q' sufficiently near p and q , we have $I(A; p, q) = I(A'; p', q')$. For any sum of arcs ΣA , each in general position relative to $D(p, q)$, we have $I(\Sigma A; p, q) = \Sigma I(A; p, q)$.

We define $I(A; p, q) = 1$ if the end-points of A are on opposite sides of $H(p, q)$ and A does not intersect $H(p, q) - D(p, q)$. We define $I(A; p, q) = 0$ if A does not intersect $D(p, q)$, or if both end-points of A are on the same side of $H(p, q)$ and A does not intersect $H(p, q) - D(p, q)$. Each arc in general position relative to the disc $D(p, q)$ can be expressed as a finite sum of arcs of these three types, $A = \Sigma A'$, and $I(A; p, q) = \Sigma I(A'; p, q)$, mod 2. It can be shown that $I(A; p, q)$ is independent of the way A is decomposed into the sum $\Sigma A'$.

We may now state our principal theorem.

INTERSECTION THEOREM: *Let A be an arc with end-points a and b contained in the metric space M . If S is a segment in A , then*

1. *S contains two points p and q such that $D(p, q)$ does not intersect the subarcs (ap) and (qb) ; distance $[(ap), (qb)] = pq$;*
2. *If S contains no equilateral triple, i.e., three points p_1, p_2 and p_3 such that $p_1p_2 = p_2p_3 = p_3p_1$, then for any such p and q we have $I(A; p, q) = 1$.*

COROLLARY 1. *If a simple closed curve C contains one subsegment S without an equilateral triple, then S contains two points p and q such that $I(C; p, q) = 1$.*

This corollary can be interpreted as a linking theorem since, in E_n , $S(p, q)$ will be an $n-2$ dimensional metric sphere, and will link C .

COROLLARY 2. *If in E_n a simple closed curve C possesses a tangent at a point p , then each segment containing p contains two points whose dual sphere links C .*

LEMMA 1. *Each simple closed curve C in any metric contains an equilateral triangle.¹*

Assume the contrary. Then $I(C; x, y)$ is defined for any x, y in C and continuous in x, y . Being an integer it is constant. But if p' and q' are two points of C whose distance is maximal then clearly $I(C; p', q') = 0$, but corollary 1, together with this, yields a contradiction.

LEMMA 2. *If T is a tripod, that is, the sum of three free arcs having one*

and the same end-point in common, then, in each metric, T contains an equilateral triangle.

Call A the sum of two of the three arcs. Assuming the Lemma false, $I(A; x, y)$ is defined for each x, y in T . It is continuous, hence is readily seen to be constant. For two points p, q close together on this third arc but not near A , we have $I(A; p, q) = 0$. The Intersection Theorem applied to A combines with this observation to yield a contradiction. These two lemmas combine to yield

THEOREM 2. *Each continuous curve which is not a simple arc contains in each metric an equilateral triple.*

A sequence of n points p_1, p_2, \dots, p_n contained in an arc A and arranged in natural order will be called a polygon on A . Similarly we may have a polygon on a simple closed curve. If $p_1 = p_n$ the polygon is called closed, and is called equilateral if $p_1p_2 = p_2p_3 = \dots = p_{n-1}p_n$.

The n -Lattice Theorem, due to Menger, states that on any arc, for each integer n , and in each metric, there exists an equilateral polygon of n sides. Menger's proof of this theorem was incomplete. Alt and Beer gave a proof for arcs contained in E_n . Later, I. J. Schoenberg gave a proof of the theorem for general arcs. Schoenberg raised the question as to whether the theorem is true for closed polygons on closed curves. As an application of our Intersection Theorem, together with the well known theory of the degree of a mapping of an $n-1$ sphere into E_n we can prove

THE CLOSED N -LATTICE THEOREM. *A simple closed curve contains, in each metric and for each positive integer n , a closed equilateral polygon of n sides.*

In conclusion we should like to mention the following open problem: Does the conclusion of Corollary 1 hold without assumption of the existence of no equilateral tripleon S ?

¹ Lemmas 1 and 2, together with Theorem 2, were conjectured by L. M. Blumenthal. The author presented a proof of these to the American Mathematical Society at the November meeting, 1942.

ON A STATISTICAL GENERALIZATION OF METRIC SPACES

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In a very interesting paper Karl Menger¹ has recently introduced a statistical generalization of semi-metric and metric spaces. According to Menger a set R of elements (points) is called a statistical semi-metric space if with each pair of points p and q of the space R a real function $F(x; p, q)$ is associated satisfying the following conditions:

1. $F(x; p, q) = 0$ for $x \leq 0$ and $\lim_{x \rightarrow \infty} F(x; p, q) = 1$.
2. $F(x; p, q)$ is a non-decreasing function of x and continuous to the left.
3. $F(x; p, q) = F(x; q, p)$ for any pair of points p and q .
4. $F(x; p, p) = 1$ for any $x > 0$.

The function $F(x; p, q)$ can be interpreted as the probability distribution function of the distance of p and q ; i.e., for any value x , $F(x; p, q)$ denotes the probability that the distance of p and q is less than x . In all that follows a distribution function will mean a function of a real variable x which satisfies conditions 1 and 2.

As a statistical generalization of the triangular inequality in metric spaces the following inequality has been proposed by Menger: For any three points p, q and r we have

$$5. \quad T[F(x; p, q), F(y; q, r)] \leq F(x + y; p, r)$$

where $T(a, b)$ is a function of two variables satisfying certain conditions. A statistical semi-metric space is called a statistical metric space if inequality 5 is satisfied for all triples, p, q and r .

Menger's generalization of the triangular inequality has the drawback that it involves an unspecified function $T(a, b)$ and one can hardly find sufficient justification for a particular choice of this function. Furthermore the notion of "between" introduced by Menger on the basis of inequality 5 has the properties of the between relationship in metric spaces only under restrictive conditions on the distribution functions $F(x; p, q)$. Here we propose another statistical generalization of the triangular inequality which is free from the above mentioned difficulties.

By the symbolic sum $F(x) \dot{+} G(x)$ of two distribution functions $F(x)$ and $G(x)$ we mean the distribution function $H(x)$ given by the Stieltjes integral

$$H(x) = \int \int d[F(u)G(v)]$$

where the integration is to be taken over the domain of the (u, v) plane

given by the inequality $u + v < x$. Thus if X and Y are independently distributed random variables with the distribution functions $F(x)$ and $G(x)$, respectively, then $F(x) \dot{+} G(x)$ is the distribution function of $X + Y$.

In all that follows for any two distribution functions F and G the symbol $F \leq G$ will mean that the inequality $F(x) \leq G(x)$ holds for all values of x . The symbol $F < G$ will mean that $F \leq G$ and F is not identically equal to G . The symbols $F \geq G$ and $F > G$ are synonymous with the symbols $G \leq F$ and $G < F$, respectively.

As a statistical generalization of the triangular inequality we propose the following inequality: For any three points p, q, r we have

$$5'. \quad F(x; p, q) \dot{+} F(x; q, r) \leq F(x; p, r).$$

We will say that two points p and q are different, in symbol $p \neq q$, if there exists a positive value x such that $F(x; p, q) < 1$. We will say that a point q lies between the points p and r if

$$6. \quad p \neq q \neq r \neq p,$$

and

$$7. \quad F(x; p, q) \dot{+} F(x; q, r) = F(x; p, r).$$

Let the symbol pqr denote the relationship that q lies between p and r . It can be shown that the "between" relationship defined here has the same properties as the between relationship in metric spaces,² i.e., our statistical between relationship satisfies the following conditions:

- I. From pqr it follows that rqp .
- II. From pqr it follows that qrp and rpq cannot hold.
- III. From pqr and prs it follows that pqs and qrs .

¹ Menger, K., *Proc. Nat. Acad. Sci.*, 28, 535 (1942). Reference is also made to a paper presented to the Mathematical Society, Notre Dame, Ind., November, 1942.

² Menger, K., *Mathematische Annalen*, 100, 77 (1928).

A SUFFICIENT CONDITION THAT A C-CHARACTERISTIC BE GEOMETRIC

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Introduction.—If $\{\gamma\} = \{\gamma_0; \gamma_1, \gamma_2, \dots, \gamma_p\}$ is the characteristic of a homaloidal net defined at a set of p points, then $\{\gamma\}$ must satisfy Cremona's equations

$$\begin{aligned} x_1^2 + x_2^2 + \dots + x_p^2 - x_0^2 &= -1 \\ x_1 + x_2 + \dots + x_p - 3x_0 &= -3. \end{aligned} \quad (1)$$

Further, if the multiplicities γ_i are ordered so that $\gamma_1 \geq \gamma_2 \geq \gamma_3 \geq \dots \geq \gamma_p$, $\{\gamma\}$ must satisfy the set of inequalities

$$\begin{aligned} \gamma_0 &\geq \gamma_1 + \gamma_2 \\ 2\gamma_0 &\geq \gamma_1 + \gamma_2 + \dots + \gamma_6 \\ 3\gamma_0 &\geq 2\gamma_1 + \gamma_2 + \dots + \gamma_7 \\ 3\gamma_0 &\geq \gamma_1 + \gamma_2 + \dots + \gamma_9 \\ &\dots\dots\dots \end{aligned} \quad (2)$$

These inequalities express the fact that no curve of order k and defined at the p points can meet the net in more than $\gamma_0 k$ points. Coble¹ has conjectured that the conditions (1) and (2) are sufficient as well as necessary.

In this paper we will show that the conditions (2) are actually sufficient. Indeed, the less stringent set of inequalities given in (5) will suffice. The notation and point of view is that used in "Cremona's Diophantine Equations."²

We begin with the proof of

LEMMA: If $c_0 > 0$ in a solution $\{c\}$ of equations (1), then $2c_0 > c_1 + c_2 + c_3$, where

$$c_1 \geq c_2 \geq c_3 \geq \dots \geq c_p \quad (3)$$

It is clear that $c_0 > c_1 \geq c_2 \geq c_3$. Let us write

$$c_1 = c_0 - a_1; \quad c_2 = c_0 - a_2; \quad c_3 = c_0 - a_3;$$

where a_1, a_2, a_3 are all positive. Substitution in (1) yields

$$\begin{aligned} c_4^2 + \dots + c_p^2 &= 2(a_1 + a_2 + a_3)c_0 - 2c_0^2 - (a_1^2 + a_2^2 + a_3^2) - 1 \\ c_4 + \dots + c_p &= (a_1 + a_2 + a_3) - 3. \end{aligned}$$

If $a_1 + a_2 + a_3 - 3 = 0$, then $a_1 = a_2 = a_3 = 1$ and it is easily verified that in this case $\{c\} = \{1; 0^p\}$ or $\{2; 1^3 0^{p-3}\}$. Both of these satisfy the conditions of the lemma.

³ Coble, A. B., "Cremona's Diophantine Equations," *Am. Jour. Math.*, **56**, 459-489 (1934).

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FURTHER OBSERVATIONS ON THE SPECIFICITY OF HYPOXANTHINE FOR PHYCOMYCES

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Communicated May 25, 1943

In a previous communication Robbins and Kavanagh¹ reported hypoxanthine to benefit the spore germination and early mycelial growth of *Phycomyces* in a basal mineral-dextrose solution containing asparagine and thiamine. The benefit was more marked in the presence of the filtrate from a potato extract (D_R fraction) which had been treated with charcoal. A comparison of the activity of hypoxanthine with other compounds led to the conclusion that the active substance must have the eighth position and the seventh or ninth position on the purine ring open for substitution, oxygen in the sixth position and hydrogen or NH_2 in the second position.

Through the courtesy of Dr. George Hitchings I have been able to test 5 additional compounds. These were 8-oxypurine, 6-amino-8-oxypurine, 6,8-dioxypurine, 7-methyl guanine and 1,7-dimethyl guanine. The effect of each of these compounds on the percentage of *Phycomyces* spores which germinated at 25°C. on a mineral-dextrose medium containing thiamine, asparagine and one per cent purified agar was compared with that of hypoxanthine and guanine. Each compound was used alone and in the presence of a D_R fraction prepared from potato tubers. A comparison was made also between the action of hypoxanthine, of guanine and of each of the five analogs on the dry weight of the mycelium produced in 48 hours at 27°C. in 10 ml. of the basal liquid medium and the same medium to which the D_R fraction was added. Each compound was used per flask or per petri dish in the following amounts: 100 $\mu\text{g.}$, 10 $\mu\text{g.}$, 1 $\mu\text{g.}$ and 0.1 $\mu\text{g.}$

The 8-oxypurine, 6-amino-8-oxypurine and 6,8-dioxypurine were ineffective. Although identical otherwise with hypoxanthine the 8-oxypurine has hydrogen in the 6th position and the 6-amino-8-oxypurine has the amino group in the 6th position. The inactivity of these two compounds agrees with the earlier observations which indicated that the active compound should contain oxygen in the 6th position. The 6,8-dioxy-

purine is identical with hypoxanthine except that the hydrogen in the 8th position in hypoxanthine is replaced by oxygen. This change rendered the 6,8-dioxypurine inactive; a result which agrees with the earlier conclusion by Robbins and Kavanagh that the 8th position must be opened for possible substitution.

It was anticipated that the two guanines also might prove to be inactive since both had the CH_3 group in the 7th position and the previous results indicated that in order to have an active compound the 7th or 9th position should be open for substitution. However, both the 7-methyl guanine and the 1,7-dimethyl guanine proved to be partially active. It is not possible to determine relative activities accurately with the methods used. It appeared, however, that the 7-methyl guanine and 1,7-dimethyl guanine had between 1/10 and 1/100 the activity of hypoxanthine.

These results support the idea that the action of hypoxanthine on *Phycomyces* has a considerable degree of specificity. Guanine is the only other compound² which has been found to approach the activity of hypoxanthine.

Pennington³ reported hypoxanthine or an equimolecular mixture of adenine and guanine to be essential for the growth of *Spirillum serpens*. Neither adenine nor guanine alone was effective and sufficient amounts of either interfered with the physiological effectiveness of hypoxanthine.

An increasing number of observations on various organisms⁴ show that the purines probably function as growth substances. There seems, however, to be little uniformity since the specific purines which are active appear to vary from organism to organism.

¹ Robbins, W. J., and Kavanagh, F., *Proc. Nat. Acad. Sci.*, **28**, 65-69 (1942).

² W. J. Robbins and F. Kavanagh found a purine, kindly furnished by Dr. D. W. Wooley, to have about one-half the activity of hypoxanthine. This compound is aspurine and is not fully defined chemically. See Wooley, D. W., *16th International Physiological Congress, Proc. II*, 126 (1938). The formula given is $\text{C}_6\text{H}_7\text{N}_5\text{O}_2$. The picrate was found to be similar in all of its physical properties to the picrate of adenine. We prepared the silver nitrate crystals of aspurine and found them to be very small, isolated and not in clusters, nearly straight and pointed. The silver nitrate crystals of aspurine treated with nitrous acid were identical in appearance with the silver nitrate crystals of hypoxanthine.

³ Pennington, D. E., *Proc. Nat. Acad. Sci.*, **28**, 272-276 (1942).

⁴ For references to earlier work, see the papers by Robbins and Kavanagh and by Pennington.

*A MUTANT OF DROSOPHILA MELANOGASTER RESEMBLING
THE SO-CALLED UNSTABLE GENES OF DROSOPHILA VIRILIS*

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In a number of papers Demerec (see 1941) described genetic phenomena in *Drosophila virilis* which he interprets as due to unstable, ever-mutating genes. The best analyzed case is that of alleles at the miniature (small wings) locus. The decisive facts are: Among a series of *mt*-alleles two called 3 and 5 showed the phenomenon in question. This means that the offspring consists of *mt* individuals, mosaics and normals, the latter breeding true, the others not. A mosaic is a fly with more or less miniature patches on normal wings, or one normal and one miniature wing (see below). Unstable *mt* appears in three well-defined "sub-alleles." Of these, *mt^b* is stable, *mt^c* produces only miniature and mosaic offspring, and *mt^a* normal, miniature and mosaic offspring. Demerec considers these facts the consequence of, in *mt^a*, instability of the *mt* gene both in somatic and germinal cells and, in *mt^c*, of instability only in somatic cells. From the size of the mosaic spots and the number of wild-type flies, it is concluded that the "mutability" begins rather late in development and the mutation is always one from miniature to normal, both in somatic and sex cells. In addition, a series of modifiers exist. One increases the number of normals (called germinal mutability) but only in the *mt^a* line, which always segregates normals. Another series of modifiers (called *S*-genes) increases only the mosaic spots (i.e., are supposed to act only upon somatic mutability). Without these enhancers a small percentage of flies show mosaic spots and these extend rarely to an entire wing. In the presence of the modifiers the percentage of mosaics is increased up to 95 per cent, and one of the *S*-genes frequently produces flies with one normal and one miniature or mosaic wing. There is, in addition, a sexual difference in all these effects. Finally, the *a*, *b*, *c* forms may change into each other and in a reversible way.

I had always expected that these important facts would, one day, find their explanation without recourse to "unstable genes." The case discovered in *Drosophila melanogaster* seems to furnish such an explanation. In the course of a study of spontaneous mutation (soon to be presented in detail) a number of alleles were found at the silver (1st chromosome) and arc (2d chromosome) loci. The silver alleles produce predominantly pointed wings and are therefore called *svr^{poi}*, *svr^{poi s}*, etc. The arc alleles are expressed, if at all, as broad and angular wings called *bran*, *bran'*, *bran^{dp}*, etc. These two loci have, in addition, a typical interaction which characterizes the different combinations of the alleles at the two loci and

some alleles can be distinguished only by this combination effect in which either the *bran* or the *poi* phenotype is more or less epistatic or a compromise form is produced. Thus *bran* homozygous with *svr^{poi}* has a soft, shortened, more or less pointed and blistered wing called soft blistered. Among these combinations one was found between *svr^{poi}* and a *bran* allele called *bran^{dp}* which simulated completely the *mt^c* case in *Drosophila virilis*. The offspring in this line, i.e., *bran^{dp}/bran^{dp}; svr^{poi}/svr^{poi}* (or hemizygous male) consists of a majority of pointed flies, a minority of flies with one wing pointed, one wing truncated (abbreviated *poi dp*) and a percentage of transitions from a pointed to a truncated wing in all conditions of asymmetry down to a symmetrical, truncated (dumpy-like) wing. All types, if selected, produce the same offspring, but modifiers for higher percentages of the not pointed wings can be selected. The constitution of this line, i.e., homozygosity of the two loci is easily tested in outcrosses as well as with different alleles of the two loci. Whenever the parental combination is recovered, and also in a few compounds of *bran^{dp}* with another *bran* allele, the characteristic variation of the phenotype appears. The homozygote *bran^{dp}/bran^{dp}*—and this is an important fact—has no visible effect if the *svr^{poi}* mutant is absent.

The first question is whether the series of phenotypes from pointed to dumpy, which we may call in brief the *poi dp* effect, can be assumed to be comparable to those described by Demerec. There is no doubt in regard to the unchanged wings, i.e., miniature in *virilis*, pointed in my case, nor for the type with one wing +, one *mt* in *virilis*, one pointed, one dumpy in my case. The decisive types are the mosaic types in *virilis* and the intermediates in my case. Demerec assumes that the mosaic spots of miniature cells are genetically miniature, and the normal wing parts are genetically normal by somatic mutation. If one thinks of such mosaics in a general way, comparing them with gynanders or mosaic spots analyzed by markers, one is led to such an interpretation. But in the case in question this is not conclusive. A miniature wing is essentially one in which cell growth after pupation is inhibited (see Dobzhansky, 1929; Goldschmidt, 1935; Waddington, 1940). If this inhibition had a narrow threshold condition so that it would act only partially in the presence of a certain genetic condition, a mosaic-like structure of the wing would appear in the varying and asymmetrical patches of miniature tissue, including also the normal and the +, *mt* wings. Actually we know of no case of a real mosaic within a wing, but many cases in which such an asymmetrical threshold effect occurs (see my discussion of the nicking effect, in Goldschmidt, 1940, p. 222 ff.). In my case the decision is between a pointed and a ≠ truncated wing. As truncation is a phenomenon at the wing edge, no mosaic in a plane can become visible. The mosaic produced by the transgressive threshold action appears in the form of a wing edge showing all transitions from pointed to dumpy,

more or less asymmetrically. Thus I believe that also in the miniature case there is no necessity for the assumption that the normal wing cells are genetically different from the miniature cells. A threshold condition acting within narrow limits can produce the apparent mosaic within the genetically miniature cells.

The data in my case indicate clearly the reason for the variable effect. The *bran^{dp}* allele, interacting with the *svr^{poi}* locus, is responsible for the threshold condition. Other *bran* alleles have a truncating effect alone and together with *svr^{poi}* produce wing types like soft blistered, truncated blistered, rudimentary blistered, etc. But *bran^{dp}* is an allele which alone has no visible effect but with *svr^{poi}* hardly succeeds in pushing the pointed wing over the threshold toward a dumpy wing. In other words, the *bran* allele acts near the level of epistasis between *bran* and pointed with the effect of a scintillating epistasis for whole wings or parts of them. In our case the explanation is clear: (1) a combination of two interacting loci, (2) the presence of one allele affecting epistasis near the threshold level; (3) the developmental physiology of the wing. As the parallelism with miniature is practically complete (including also modifiers and sexual difference), I conclude that *mt^c* collaborates with another allele which, like *bran^{dp}*, has no visible action alone, but, if epistatic, pushes wing cell development toward normal just as *bran^{dp}* pushes it towards truncation, and, further, that *mt^c* is not an *mt* allele but ordinary *mt* in the presence of the other locus, say *e*, which has an epistatic action near the threshold, but no visible effect alone.

In my case the location of *bran^{dp}* in the arc region is clear because alleles with visible action exist. In Demerec's case the second locus *e* is difficult to locate because it also had no action alone, and no alleles with visible effect have been reported. It might even be linked with the miniature locus, as suggested by some data. But there are no general difficulties to an understanding of *mt^c* without unstable genes. We turn now to *mt^a*. Here another group of facts found in my case comes in. It turned out that within the *poi dp* stock an allele of *bran* is present occasionally, called *bran'*, which together with *svr^{poi}* produces a wing with a phenotype like rudimentary and blistered. This combination is less viable and, if produced by mating *bran^{dp}/bran'*, appears in less than a quarter of the expected number. The allele *bran'* alone produces a kind of truncated wing. The segregating *bran'/bran'*; *svr^{poi}* flies breed true. Returning to our former comparison of the two cases, the *svr^{poi}* locus paralleled the *mt* locus in virilis; *bran^{dp}* paralleled an unknown locus in virilis with the discussed effect upon epistasis, otherwise producing a normal wing. If we had now in addition another allele of the latter (called *e'*) which if homozygous produces complete epistasis of its normal effect over the *mt* effect, thus paralleling the action of *bran'*, a true breeding normal fly would result from this combination *mt/mt; e'/e'*. A line containing *e* and *e'* with a chance for segregation

of e'/e' would be the complete explanation for Demerec's mt^a , throwing the "mutation" to normal. There is one detail, however, which cannot be explained as long as the detailed data on the virilis case are not available, namely, the normal constitution of the mt^a line in regard to e' and e . What I termed e' may be more than one locus with complicated segregation in addition to the possibility of linkage with mt . The results of outcrosses as well as the details of the so-called reversible changes of mt^a into b , c , and vice versa, ought to furnish this information. I do not doubt that a re-check of the facts, abandoning the idea of the unstable gene and trying to apply the interpretation of my so similar case would result in a simple explanation of all details in terms of factor interaction, epistasis and threshold conditions.

It would be interesting to try to apply this interpretation also to the cases of variegation in plants, though there is the basic difficulty that nothing is known of developmental genetics of color, which prevents a discussion of the possibility of a purely phenotypical threshold effect. The most interesting case of this kind is the one beautifully analyzed by Rhoades. Looking at his data I am struck again by the great parallelism to my case: A homozygous condition for colorless is made to produce mosaics by the recombination with another mutant Dt which alone has no visible effect at all. Thus we have the parallel: aa (colorless) parallels svr^{poi} , Dt parallels $bran^{dp}$. Rhoades himself emphasizes that up to this point unstable genes are not needed as an explanation. But he thinks to have proven his point by finding that from colored or mosaic anthers present in a stock made up by outcrossing, pollen may be derived which acts either as aa or as heterozygous for a and a color allele (A). This again parallels completely my case when the allele $bran'$ is present which then, here, would be represented by a new Dt allele which produces color with the tester allele of a and which seems to have been introduced when making up the stock with spotted anthers which required a special constitution. The parallel is so close that a test may be suggested which should be easy to perform in the maize stock of Rhoades. In the published account I cannot find results which exclude such an interpretation, but the decisive tests would still have to be made, e.g., numerous crosses with the tester stock and pollen from green anthers in the mosaic stock which gave the "mutants" from colored anthers.

Demerec, M., *Cold Spring Harbor Symposia*, 9, 145-149 (1941). Here quot. of former papers.

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A TABLE DETERMINING L.D.50 OR THE FIFTY PER CENT END-POINT

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Communicated June 12, 1943

It has been shown¹ that if we may use the growth curve in bio-assay and if we have three proportions P_1, P_2, P_3 of the population affected by doses D, Dr, Dr^2 of some biological, the values of the parameters γ and α of the curve may be obtained in terms of the two simple combinations

$$A = 2(P_1 + P_2 + P_3) - 3 \quad \text{and} \quad B = P_3 - P_1 \quad (1)$$

of these proportions provided the number n of animals used at each dosage be the same. It is therefore possible to give a double entry table which states the values of " γ " = γ/c and of " α " = $c\alpha$, where $c = \log r$, and of their standard deviations as computed by the formulae of R. A. Fisher² for $n = 1$: to obtain the standard deviations for any value of n it is merely necessary to divide their tabulated values by \sqrt{n} .

In the table the values of B go by intervals of 0.05 from 0.30 to 0.95, inclusive. Because the standard deviations of γ are large for small values of B , the table is not carried below $B = 0.30$. The values of A are given by intervals 0.1 from 0 to the highest value possible for the given values of B , which ranges from $A = 1.0$ for $B = 0.95$ to $A = 2.3$ for $B = 0.30$. If n is 20 or any divisor thereof the values of " γ " and " α " and their standard deviations (for $n = 1$) can be read directly from the table; for other values of n , interpolation is necessary. The table is restricted to positive values of A and B ; for negative values of either or both, the numerical values of " γ " and " α " will be in the table but signs of " γ " or " α " or both will have to be taken in accordance with this scheme:

	$A > 0$	$A < 0$
$B > 0$	γ neg., α pos.	γ pos., α pos.
$B < 0$	γ pos., α neg.	γ neg., α neg.

Bacteriologists will prefer to work in terms of dilutions $1:a, 1:ar, 1:ar^2$ rather than dosages. Dilutions and dosages are reciprocals, which means that as logarithms are used each is the negative of the other. To use the table to obtain that dilution $1:\delta$ which for the amount of the biological administered affects half the population one has merely to take

$$\log \delta = \log ar - "\gamma" \log r \pm \sigma("\gamma") \log r \quad (2)$$

and convert to antilogarithms. This can be illustrated on an example more clearly than stated in words.

VALUES FOR "γ" = γ/c AND "α" = cα FOR SPECIFIED VALUES OF A AND B AND FOR THEIR STANDARD DEVIATIONS IF n = 1

B		0.65	0.70	0.75	0.80	0.85	0.90	0.95
A	1.4 "γ"	-0.74 ± 0.68	-0.73 ± 0.58	-0.73 ± 0.51				
	"α"	1.30 ± 1.56	1.58 ± 1.87	2.04 ± 2.51				
	1.5 "γ"	-0.78 ± 0.61	-0.78 ± 0.52					
	"α"	1.48 ± 1.83	1.92 ± 2.47					
	1.6 "γ"	-0.83 ± 0.53						
	"α"	1.81 ± 2.44						
B		0.30	0.35	0.40	0.45	0.50	0.55	0.60
A	0 "γ"	0 ± 1.92	0 ± 1.65	0 ± 1.44	0 ± 1.28	0 ± 1.15	0 ± 1.05	0 ± 0.96
	"α"	0.31 ± 0.74	0.37 ± 0.76	0.42 ± 0.77	0.48 ± 0.79	0.55 ± 0.82	0.62 ± 0.84	0.69 ± 0.88
	0.1 "γ"	-0.11 ± 1.94	-0.10 ± 1.66	-0.09 ± 1.44	-0.08 ± 1.28	-0.07 ± 1.15	-0.07 ± 1.05	-0.06 ± 0.96
	"α"	0.31 ± 0.74	0.37 ± 0.76	0.42 ± 0.77	0.48 ± 0.79	0.55 ± 0.82	0.62 ± 0.84	0.69 ± 0.88
	0.2 "γ"	-0.23 ± 1.98	-0.20 ± 1.68	-0.18 ± 1.46	-0.16 ± 1.29	-0.15 ± 1.16	-0.13 ± 1.05	-0.13 ± 0.96
	"α"	0.31 ± 0.74	0.37 ± 0.76	0.43 ± 0.77	0.49 ± 0.79	0.55 ± 0.82	0.62 ± 0.85	0.70 ± 0.89
A	0.3 "γ"	-0.34 ± 2.04	-0.30 ± 1.71	-0.26 ± 1.48	-0.24 ± 1.30	-0.22 ± 1.16	-0.20 ± 1.05	-0.19 ± 0.96
	"α"	0.31 ± 0.75	0.37 ± 0.76	0.43 ± 0.78	0.49 ± 0.80	0.56 ± 0.82	0.63 ± 0.85	0.70 ± 0.90
	0.4 "γ"	-0.45 ± 2.12	-0.39 ± 1.76	-0.35 ± 1.51	-0.31 ± 1.32	-0.29 ± 1.17	-0.27 ± 1.05	-0.25 ± 0.96
	"α"	0.32 ± 0.75	0.37 ± 0.76	0.43 ± 0.78	0.50 ± 0.80	0.56 ± 0.83	0.63 ± 0.86	0.71 ± 0.90
	0.5 "γ"	-0.56 ± 2.22	-0.49 ± 1.82	-0.43 ± 1.54	-0.39 ± 1.34	-0.36 ± 1.18	-0.33 ± 1.06	-0.31 ± 0.95
	"α"	0.32 ± 0.76	0.38 ± 0.77	0.44 ± 0.79	0.50 ± 0.81	0.57 ± 0.84	0.64 ± 0.87	0.72 ± 0.92
A	0.6 "γ"	-0.67 ± 2.33	-0.58 ± 1.89	-0.51 ± 1.58	-0.47 ± 1.36	-0.43 ± 1.19	-0.40 ± 1.06	-0.37 ± 0.95
	"α"	0.32 ± 0.76	0.38 ± 0.78	0.44 ± 0.80	0.51 ± 0.82	0.58 ± 0.85	0.66 ± 0.88	0.74 ± 0.93
	0.7 "γ"	-0.77 ± 2.44	-0.67 ± 1.94	-0.59 ± 1.61	-0.54 ± 1.37	-0.49 ± 1.20	-0.46 ± 1.06	-0.43 ± 0.95
	"α"	0.33 ± 0.77	0.39 ± 0.79	0.45 ± 0.81	0.52 ± 0.83	0.59 ± 0.86	0.67 ± 0.90	0.76 ± 0.95
	0.8 "γ"	-0.87 ± 2.54	-0.76 ± 2.01	-0.67 ± 1.65	-0.61 ± 1.39	-0.56 ± 1.20	-0.52 ± 1.06	-0.49 ± 0.94
	"α"	0.34 ± 0.78	0.40 ± 0.80	0.46 ± 0.82	0.53 ± 0.85	0.61 ± 0.88	0.69 ± 0.92	0.78 ± 0.98
A	0.9 "γ"	-0.97 ± 2.64	-0.84 ± 2.06	-0.75 ± 1.67	-0.67 ± 1.40	-0.62 ± 1.20	-0.57 ± 1.05	-0.54 ± 0.93
	"α"	0.34 ± 0.79	0.41 ± 0.81	0.48 ± 0.83	0.55 ± 0.86	0.62 ± 0.90	0.71 ± 0.95	0.81 ± 1.01

Suppose that we have 20 guinea pigs inoculated with dilutions 1:4, 1:8, 1:16 of a standard toxoid and also of one to be tested against it, that subsequently a dose of the toxin is given, and finally the number of survivors be recorded as follows:³

STANDARD				TO BE TESTED		
DILUTION	<i>n</i>	SURVIVALS		<i>n</i>	SURVIVALS	
1:4	20	20	$P_3 = 1.00$	20	15	$P'_3 = 0.75$
1:8	20	15	$P_2 = 0.75$	20	6	$P'_2 = 0.30$
1:16	20	8	$P_1 = 0.40$	20	1	$P'_1 = 0.05$

From (1) we have $A = 1.30$, $B = 0.60$, $A' = -0.80$, $B' = 0.70$, with $r = 2$ and $\log r = 0.301$.

From the table, entering with $A = 1.30$ and $B = 0.60$, and neglecting for the moment the standard deviations we find " γ " = -0.73 . Hence from (2) with $ar = 8$ for the middle dilution

$$\log \delta = \log 8 - (-0.73)(0.301) = 0.903 + 0.22 = 1.123, \quad \delta = 13.3.$$

The 50% end-point is at dilution 1:13.3. For the biological to be tested $A' = -0.80$ is negative which means that " γ " must be positive and equal to 0.44. Hence, still neglecting the standard deviation both in the table and in (2),

$$\log \delta' = \log 8 - (0.44)(0.301) = 0.903 - 0.13 = 0.773, \quad \delta = 5.9.$$

The strength of the toxoid to be tested is thus only $5.9/13.3 = 0.44$ or 44% of that of the standard at the L.D.50 points.

To discuss the significance of the ratio it is best to test the significance of the differences of the values of $\log \delta$ and $\log \delta'$ as given in (1). The standard deviation of " γ " in the table for $n = 1$ is entered as 0.83 and for " γ " as 0.77. Dividing by $\sqrt{20}$ since $n = 20$ we have 0.19 and 0.17, respectively. Then multiplying by $\log r$ we find finally

$$\begin{aligned} \log \delta &= 1.123 \pm 0.057, & \log \delta' &= 0.773 \pm 0.051 \\ \text{diff.} &= -0.35 \pm \sqrt{(0.057)^2 + (0.051)^2} = -0.35 \pm 0.076. \end{aligned}$$

The difference is therefore highly significant.⁴

In comparing these two biologicals the L.D.50 points have been used. If the comparison is to remain valid at other points, the values of α must be nearly enough alike so that the difference between them may be ignored. In this particular case " α " = 1.00 and $\alpha = \alpha'/c = 1.00/0.301 = 3.32$; further, $\sigma(\alpha') = 0.28$ for $n = 20$ and $\sigma(\alpha) = 0.93$ so that $\alpha = 3.32 \pm 0.93$. The value of α' would not be significantly different from that of α unless it were outside the limits 5.18 and 1.46 which are rather wide open limits. As a matter of fact α' in the illustration is the same as α and the standardization is valid over the whole range.⁵

It sometimes happens that animals are lost during the experiment so that one cannot be sure whether they would have been survivors or not. Suppose, for example, that in the group treated with the standard biological we had had at 1:16 one animal lost, 7 survivors and 12 deaths. If the one lost be taken as surviving we have $\delta = 13.3$ as before, but if it were taken as not surviving we should have $P_1 = 0.35$, $A = 1.2$, $B = 0.65$ which would give " γ " = -0.66 and $\delta = 12.7$ which cannot be considered a significant change; but what we know is that $\delta = 12.7$ or 13.3 . The change in α would also be well under the sampling allowance; and furthermore the standard deviations whether of γ or α would not be much different on the two different hypotheses.

¹ See these PROCEEDINGS, 29, 79-85, 114-120, 150-154 (1943).

² These formulae assume that the numbers are large and may therefore give inaccurate values for the standard deviations when the numbers are as small as they often are in the case of bio-assays; but some estimate of standard deviations seems better than none provided the estimates are used with discretion.

³ It will be observed that we have used P_3 for the lowest dilution (largest dose) and P_1 for the highest dilution (smallest dose) which is in accord with our previous convention that P_3 represented the greatest effect, which in this case is a protective effect.

⁴ If we take the limits specified by the standard deviation itself, we have -0.426 and -0.274 which correspond to ratios of 0.38 and 0.53 in place of the value 0.44 ; if we should allow twice the standard deviation we should have limits of -0.502 and -0.198 which correspond to ratios of 0.31 and 0.63 . We cannot safely assign probabilities of 0.32 and 0.05 for the ratio being outside these respective pairs of limits, but that is always the case with the use of the standard deviation to estimate significance when the true distribution of the ratio of the variable to its standard deviation is unknown.

⁵ If two biologicals have a ratio of strength of R when compared at their 50% end-points, the ratio of strength R_P when compared at any percentage P will be $R_P = Re^Z$, where $Z = 2.65 \left(\frac{1}{\alpha''} - \frac{1}{\alpha} \right) \log \frac{P}{Q} \log_{10} r$. For comparison well away from the L.D.50, e^Z may differ considerably from 1 even if " α " and " α'' " do not differ significantly.

GROUPS CONTAINING FOUR AND ONLY FOUR NON-INVARIANT SUBGROUPS

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If a group G contains four and only four non-invariant subgroups these subgroups are transformed under G according to a permutation group of degree 4. We shall begin with the case when this permutation group is transitive. It could not be either the symmetric or the alternating group of degree 4 since each of these groups contains more than four non-invariant subgroups and to each of these subgroups there would correspond a non-invariant subgroup of G . This is impossible since G is supposed to contain only four non-invariant subgroups. These subgroups could not be transformed under G according to the octic group because this group itself contains four non-invariant subgroups and these are not transferred transitively under the octic group while the four non-invariant subgroups of G are assumed to be transformed transitively under G . Hence G would have to contain more than four non-invariant subgroups, which is contrary to the hypothesis.

It remains to consider the case when the four conjugate subgroups of G are supposed to be transformed under G according to one of the two regular permutation groups of degree 4 and hence the invariant subgroup of G which corresponds to the identity of this regular group contains the four non-invariant subgroups of G invariantly and is itself either Hamiltonian or abelian. In the former case it could not be the quaternion group since this group does not involve four similar subgroups. Its order could not be a power of 2 since it would then be the direct product of the quaternion group and the abelian group of type 1^m and hence it would involve the abelian group of type $1^m + 1$ as a characteristic subgroup.¹ Not all the subgroups of this characteristic subgroup could be invariant under G since the non-invariant subgroups of G are supposed to be conjugate under G . For similar reasons the order of the Hamiltonian group could not be divisible by two distinct prime numbers.

If G would contain exactly four non-invariant subgroups and would transform them according to the non-cyclic regular permutation group of degree 4 then G would involve a subgroup H of index 2 which would have a commutator subgroup of order 2 and an invariant abelian subgroup of index 4 under G . All the operators of H which would not be contained in this abelian subgroup would separately generate the commutator subgroup of H . Since another subgroup of index 2 under G would contain a different commutator subgroup there would be more than four non-invariant sub-

groups in G . This method of proof applies also to the case when the four non-invariant subgroups are supposed to be transformed according to the regular cyclic permutation group of order 4. It therefore results that *when G contains exactly four non-invariant subgroups it must transform them according to an intransitive group.*

It remains to consider the groups which separately contain four and only four non-invariant subgroups such that these subgroups are conjugate in pairs under the group. These subgroups are transformed under G according to an intransitive group of degree 4 whose order is either 2 or 4. In the former case G contains an invariant subgroup of index 2 which corresponds to the identity of the group of transformation of its four non-invariant subgroups. As this subgroup contains no non-invariant subgroup it is either abelian or Hamiltonian. In the former case G may be the direct product of a group of prime order and an arbitrary group in the infinite system of groups which is composed of groups which separately involve two and only two non-invariant subgroups. This is obviously the only case in which the order of the abelian subgroup of G which corresponds to the identity in the group of transformation of its four non-invariant subgroups has an order which is divisible by as many as two distinct prime numbers.

When this order is divisible by only one prime number the order of G is a power of 2 and the given abelian subgroup of G which corresponds to the identity in the group of transformations of its four non-invariant subgroups and is of index 2 under G must have exactly three independent generators one of which is of order 2. At least one of the other generators of this invariant subgroup must have an order which exceeds 2 and hence the order of G is at least 32. Each of the four non-invariant subgroups of G may be of order 2. Each of the groups in this infinite system of groups contains a commutator subgroup of order 2 which is generated by its operator of largest order. We therefore arrived at the following theorem: *There are two infinite systems of groups which are separately composed of groups such that each of them contains four and only four non-invariant subgroups which it transforms according to the intransitive group of degree 4 and of order 2. The orders of the groups in one of these systems are a power of 2 while the orders of those in the other system are a power of 2 multiplied by an arbitrary odd prime number.*

It remains to consider the possible groups which separately contain four and only four non-invariant subgroups and transform them according to the intransitive permutation group of degree 4 and of order 4. Such a group G contains two subgroups of index 2 which separately transform each of two non-invariant subgroups into itself and the other two among themselves. These two subgroups have a subgroup of index 4 under G in common which is invariant under G since each of the two given subgroups is invariant under G . Hence G contains also a subgroup of index 2 which transforms

the four non-invariant subgroups in pairs and involves the given invariant subgroup of index 4. This invariant subgroup of index 2 involves no non-invariant subgroup of G and hence it is either abelian or Hamiltonian.

When this subgroup of index 2 is the cyclic group of order 4 then G is the octic group and when it is the cyclic group of order 8 then G is the dicyclic group of order 16. When it is the abelian group of order 8 and of type 2, 1 then G is the group of order 16 obtained by extending this abelian group of order 8 by an operator of order 4 which transforms into their inverses all the operators of this abelian group of order 8 and has for its square an operator of order 2 which is not the commutator of this order. It will be proved that these three groups include all those which satisfy the conditions under consideration. In fact, in such a group the given subgroup of index 2 could clearly not be Hamiltonian. If it is abelian it cannot have more than two independent generators since the remaining operators of the group must transform each of its subgroups into itself. If it is cyclic the remaining operators of the group transform its operators according to one of the three operators of order 2 in its group of isomorphisms. Hence there results the theorem: *There are three and only three groups which separately contain four and only four non-invariant subgroups and transform them according to the intransitive group of degree 4 and of order 4.*

In closing we may direct attention to the fact that if a group G contains five and only five non-invariant subgroups they cannot be transformed under G according to an intransitive permutation group. Such a group could not be a 3, 1 isomorphism between the symmetric group of degree 3 and the group of order 2 since the permutations which are not found in the invariant subgroup of order 3 could not transform the two conjugate subgroups into each other. It could not be the cyclic group of order 6 since the permutation of order 6 could not be non-commutative with each of the non-invariant subgroups of G . As it could also not be the group of order 12 and degree 5 it results that *whenever a group contains five non-invariant subgroups and all its other subgroups are invariant then its non-invariant subgroups appear in a single set of conjugates under the group.*

¹ Cf. G. A. Miller, *Collected Works*, 1, 266 (1935).

ON INFINITE DIMENSIONAL LINEAR SPACES

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The study of infinite dimensional linear spaces² seems to be most fruitful when the spaces considered are more than abstract linear spaces; that is, when further structure has been added by introducing some undefined notion such as a topology, an inner product or a partial ordering. It is the purpose of this note to describe the results of introducing such structure in still another way.

Let us begin by observing that the theory of the classification of abstract linear spaces is quite simple. It is well known that in any linear space there exists a Hamel basis; that is, a set of linearly independent elements whose finite linear combinations constitute the whole space and Löwig³ has shown that any two Hamel bases for the same space have the same cardinal number. Calling this number the dimension of the space, it is clear that two linear spaces are isomorphic if and only if they have the same dimension and that given any cardinal number there is a linear space with this number as its dimension.

Since most of the commonly studied linear spaces have the same dimension, namely, C , one may, to a large extent, regard the different topological linear spaces, linear lattices, etc., as obtained from a single linear space by introducing different topologies, different partial orderings, etc. This point of view becomes rather suggestive in the case of normed linear spaces because of a theorem of Fichtenholz⁴ to the effect that two norms in the same linear space give the same topology if and only if the same linear⁵ functionals are continuous with respect to both of them. Thus if X is an abstract linear space of dimension C and X^* is the linear space of linear functionals on X it is clear that there is a natural one-to-one correspondence between an important class of normable topological linear spaces and a certain class of subspaces of X^* .

The method of introducing structure with which this note is concerned is suggested in two different ways by the considerations of the preceding paragraph. First, since each norm topology in X uniquely determines and is uniquely determined by the family of linear functionals which are continuous with respect to it, the structure in X obtained by introducing such a topology is identical with that obtained by *distinguishing a certain subspace of X^** . Secondly, if one asks what subspaces of an X^* can appear as "norm sets" one is led to seek means of differentiating between subspaces of X^* 's. Although the problem of classifying the subspaces of an abstract linear space is as trivial as that of determining all abstract linear spaces,

this is not the case for the norm set characterization problem. X^* is not merely an abstract linear space but has added structure by virtue of being a space of linear functionals. Furthermore, and this is what is of interest here, this structure may be regarded as induced in X^* by *distinguishing a certain subspace of X^{**}* . In precisely what sense this is true will appear below. If X is any linear space we shall call the result of distinguishing a subspace L of X^* the *linear system X_L* .

It is easy to show that, setting aside a few uninteresting exceptions, for every linear system X_L there is a topology in X with respect to which X is a topological linear space and the continuous linear functionals are precisely the members of L . Hence linear systems are not strictly speaking new mathematical objects and our method of introducing structure is at most a new *method*. However it does lead to a rather unconventional point of view, two features of which we now describe.

We begin with some definitions. Two linear systems X_L and X_M will be said to be *isomorphic* if there exists an isomorphism T between X and Y as abstract linear spaces such that m is in M if and only if there is an l in L with the property that $m(T(x)) = l(x)$ for all x in X . An isomorphism of a linear system with itself will be called an *automorphism*. If x is a member of a linear space X and L is a subspace of X^* then the member f of L^* such that $f(l) = l(x)$ for all l in L will be called the *member of L^* associated with x* . In terms of these the following easily proved theorem may be stated. Let X be a linear space and let L and M be subspaces of X^* . Let X_X^* be the linear system obtained from X^* by distinguishing those members of X^{**} associated with members of X . Then the linear systems X_L and X_M are isomorphic linear systems if and only if there is an automorphism of the linear system X_X^* which carries L into M .

This theorem justifies the statement about the structure of X^* made above and tells us that the problem of classifying the linear systems definable on a fixed linear space is identical with the problem of classifying the subspaces of a certain linear system. Thus methods which occur naturally in one problem may be applied to the other and every definition which we may make differentiating subspaces of systems of the form X_X^* carries with it a definition differentiating linear systems and conversely.

The other feature is a natural conjugacy relation that exists between linear systems. If X_L is a linear system one may consider the linear system whose linear space is L and whose distinguished functionals are those associated with members of X . This linear system will be called the *conjugate* of X_L . It is easy to see that a linear system X_L is isomorphic to the conjugate of its conjugate if and only if L is total. Such a linear system will be said to be *regular*. It should be pointed out that the conjugate of the linear system of a normed linear space is not isomorphic to the linear system of the conjugate space unless the normed linear space is reflexive and

the linear system of any normed linear space is regular whether the space is reflexive or not.

The systematic study of linear systems centers around two principal notions each of which is an obvious generalization of a corresponding notion in the theory of normed linear spaces. The first of these is that of closure of a subspace. Let X_L be a linear system. For each subspace M of $X(L)$ let M' denote the set of all l in L (x in X) such that $l(x) = 0$ for all $x(l)$ in M . If M is a subspace of X then M'' will be a subspace of X which contains M . We call it the *closure* of M and call the subspaces of X which coincide with their closures the *closed* subspaces of the system X_L . The family of closed subspaces of a linear system X_L forms a complete lattice under partial ordering by inclusion. The operation $'$ sets up a dual isomorphism between this lattice for X_L and that for its conjugate system. If X_L is regular every finite dimensional subspace of X is closed and the lattice of all closed subspace as an abstract lattice determines X_L up to an isomorphism.⁶ The lattice of X_L is modular if and only if whenever M and N are both closed subspaces of X the smallest subspace $M + N$ which contains both M and N is also closed. The notion of disjointness of subspaces of a Banach space introduced by Kober⁷ and by Lorch⁸ readily generalizes to linear systems and it is possible to prove that the lattice of X_L is modular only if any two closed subspaces with only zero in common are disjoint. Applying these considerations to normed linear spaces using a construction suggested by P. Erdős and S. Kakutani it may be shown that the lattice of closed subspaces of a normed linear space is modular if and only if the space is finite dimensional.

There are several useful facts about automorphisms of linear systems which may be established with the aid of the notion of closed subspace. For example, it is quite easy to show that if X_L is a regular linear system and x_1, x_2, \dots, x_n and y_1, y_2, \dots, y_n are two finite sets of linearly independent members of X then there is an automorphism of X_L carrying each x_i into y_i . Thus in particular the group of automorphisms of X_L is transitive on the non-zero elements of X . It follows also that if M and N are two finite dimensional subspaces of X , then there is an automorphism of X_L carrying M into N if and only if M and N have the same dimension, and that if M and N are closed subspaces of X_L , such that X/M and X/N are finite dimensional, then such an automorphism exists if and only if X/M and X/N have the same dimension. An immediate consequence of this last remark is the following theorem about normed linear spaces. Let X be a normed linear space. Then X has all of the following properties if it has any one of them. (a) X is isomorphic to its direct product with a one dimensional normed linear space. (b) X is isomorphic to one of its maximal closed subspaces. (c) X is isomorphic to its direct product with any finite dimensional normed linear space. (d) X is isomorphic to each of its

closed subspaces which has a finite dimensional quotient space. This result suggests that spaces with one and hence all of these "stability" properties might be interesting to study.

The greater part of our results are concerned with our second principal notion, that of boundedness. If X_L is a linear system a subset A of X will be said to be a *bounded* subset of X_L if for each l in L the set of real numbers of the form $l(x)$, where x runs through A , is a bounded set of real numbers. This notion leads at once to a new sort of closure for subspaces of linear systems of the form X_X^* . Let X be a linear space and let L be a subspace of X^* . The set of all members of X^* which carry bounded subsets of X_L into bounded sets of real numbers is a subspace which contains L . We call it the *bounded closure* of L and denote it by \bar{L} . If $L = \bar{L}$ we say that L is *boundedly closed*. It is readily verified that the bounded closure of any subspace is itself boundedly closed. Examples of boundedly closed subspaces of an X_X^* include all closed subspaces, all \aleph_0 dimensional subspaces, and all norm sets. It is easy to show that the intersection of any number of boundedly closed subspaces of an X_X^* is again boundedly closed. On the other hand it is not known whether or not the bounded closure of $M \dot{+} N$ follows from that of M and N . If M is finite dimensional, if M and N are both norm sets or if M is a norm set and N is \aleph_0 dimensional then $M \dot{+} N$ is boundedly closed, but only the last of these theorems is easy. The natural question as to whether or not X is a boundedly closed subspace of $X_{X^*}^{**}$ can be shown to be completely equivalent to a certain problem in measure theory which has been partially solved by Ulam⁹. More particularly, X is a boundedly closed subspace of $X_{X^*}^{**}$ if and only if the dimension \aleph of X is such that any countably additive measure function defined on all subsets of a class of cardinal \aleph taking on only the values 0 and 1 and vanishing at points is identically zero.

Let \bar{x} be a member of the linear system X_L and let $\{x_n\}$ be a sequence of members of X_L . If there exists a sequence of positive real numbers $\{\gamma_n\}$ converging to zero such that $\{(x_n - \bar{x})/\gamma_n\}$ is a bounded subset of X_L we say that $\{x_n\}$ converges to \bar{x} . The notion of convergence so defined plays an important role in the discussion of bounded closure. There are several theorems connecting the two notions some of which appear to be indispensable in proving certain theorems about bounded closure alone. By regarding the regular linear system X_L as a subsystem of the linear system L_L^* and considering the closedness of X in L^* with respect to the convergence of sequences, one is led to a notion of completeness for linear systems. If X_L is the linear system of a normed linear space then X_L is complete if and only if the normed linear space is complete and if X_L is an arbitrary regular linear system then X_L is complete if and only if every total subspace of L contains L in its bounded closure.

A classification of subspaces of linear systems of the form X_X^* which in a

sense supplements that given by the notion of bounded closure is suggested by a study of the nature of the boundedness in the linear systems X_L which they define. Let X be a linear space, let L be a subspace of X^* and consider the bounded sets in the linear system X_L . If there is a bounded subset of X_L which is contained in no proper subspace of X we say that L is *relatively bounded*. If there is a countable family of bounded subsets of X_L such that no proper subspace of X contains them all we say that L is *almost relatively bounded*. If for every countable family of bounded subsets $\{B_n\}$ of X_L there is a sequence $\{\gamma_n\}$ of positive real numbers such that $(B_1/\gamma_1) \cup (B_2/\gamma_2) \cup (B_3/\gamma_3) \cup \dots$ is a bounded subset of X_L we say that L has the *first countability property*. If there exists a countable family of bounded subsets $\{B_n\}$ of X_L such that for every other bounded subset B there is a B_n and a positive real number γ such that B/γ is contained in B_n we say that L has the *second countability property*. If the sequence $\{B_n\}$ in the immediately preceding definition may be replaced by a single bounded set we say that L is *simple*. Every simple L has the other four properties listed above. Moreover there are enough implications between the properties to assure us that of the 32 disjoint classes into which they divide the L 's at most eight are not empty. We have examples of L 's in seven of these.

There are a number of theorems describing the behavior of L 's with various of the above properties. Typical of these are the following. L is relatively bounded if and only if it is contained in a norm set. If L is relatively bounded then there is an automorphism T^* of the linear system X_X^* such that $L \cap T^*(L) = 0$. If L has the first countability property then every maximal subspace of L also has the first countability property.

Let X_L be a linear system. We say that a subset A of X is *uniformly bounded* if for each bounded subset B of the conjugate system $\text{l.u.b.}_{(1, B)} (\text{l.u.b.}_{(x, A)} l(x)) < \infty$. There always exists a subspace M of X^* such that the uniformly bounded subsets of X_L are identical with the bounded subsets of X_M . M is not unique but our proof of its existence leads to a unique result which we call the *reach* of L . If every bounded subset of X_L is uniformly bounded we say that X_L is a *uniform linear system* and that L is *uniform*. It is not known whether or not the bounded closure of a uniform L is uniform, nor whether or not the operation of taking the reach is idempotent. Among the things that may be said about uniformity are the following. Every complete linear system is uniform. The linear system of a normed linear space X need not be uniform but will be if X is of the second category or is such that X^c/X is finite dimensional where X^c denotes the completion of X . Every relatively bounded uniform linear system is simple.

By making use of the general theory of linear systems one can obtain information about the nature of norm sets. In doing this it is convenient to introduce a concept slightly more general than that of norm set. A real

valued function defined on a linear space which has all of the properties of a norm except that it may be zero at non-zero elements of the space we call a *pseudo-norm*.¹⁰ The set of all linear functionals continuous with respect to a pseudo-norm we call a pseudo-norm set. A pseudo-norm set is a norm set if and only if it is total. Among our theorems on pseudo-norm sets are the following. If L_1, L_2, \dots is a sequence of pseudo-norm sets then $L = L_1 \dot{+} L_2 \dot{+} \dots$ is bounded closed but is a pseudo-norm set if and only if for some n , $L = L_1 \dot{+} L_2 \dot{+} \dots \dot{+} L_n$. The intersection of a finite number of pseudo-norm sets is again a pseudo-norm set and if the underlying linear space is infinite dimensional, any pseudo-norm set is the intersection of two norm sets. A subspace of an X^* is an intersection of pseudo-norm sets if and only if it is relatively bounded and boundedly closed. Such a subspace need not be a pseudo-norm set. On the other hand if X_L is a complete regular linear system then L is a norm set if and only if it is relatively bounded and boundedly closed. The dual of the above theorem on sequences of pseudo norm sets is not true. However, by using uniform boundedness a notion of *regular inclusion* may be introduced in terms of which the following true analog may be stated. If L_0 is a total subspace of an X^* and L_1, L_2, \dots is a sequence of norm sets each of which contain L_0 regularly then $L = L_1 \cap L_2 \cap \dots$ is a norm set if and only if for some n , $L = L_1 \cap L_2 \cap \dots \cap L_n$.

In a subsequent note we shall describe the results we have obtained by applying the theory of linear systems to the study of convex topological linear spaces. The proofs of the theorems announced in these notes as well as those of a good many others will appear in two longer papers to be published later.

¹ This note is a highly condensed résumé of the major portion of the author's doctoral thesis, Harvard, 1942.

² By a linear space we mean a real linear space. See Banach, *Théorie des opérations linéaires*, 1932, p. 26.

³ Löwig, H., "Über die Dimension linearer Räume," *Studia Mathematica*, 5, 18-23 (1934).

⁴ Fichtenholz, G., "Sur les fonctionelles linéaires, continues au sens généralisé," *Rec. Math. (Mat. Sbornik) N. S.*, 4, 192-213 (1938).

⁵ In this note linear means additive and homogeneous.

⁶ Cf. Mackey, G. W., "Isomorphisms of Normed Linear Spaces," *Ann. Math.*, 43, 244-260 (1942).

⁷ Kober, "A Theorem on Banach Spaces," *Compositio Mathematica*, 7, 135-140 (1939).

⁸ Lorch, E. R., "On a Calculus of Operators in Reflexive Vector Spaces," *Trans. Amer. Math. Soc.*, 45, 217-234 (1939).

⁹ Ulam, S., "Zur Masstheorie in der allgemeinen Mengenlehre," *Fund. Math.*, 16, Satz 1 146 (1930).

¹⁰ Not to be confused with the pseudo-norm of Hyers; Hyers, D. H., "A Note on Linear Topological Spaces," *Bull. Amer. Math. Soc.*, 44, 76-80 (1938). Cf., however, Wehausen, J. V., "Transformations in Linear Topological Spaces," *Duke Math. Jour.*, 4, 157-169 (1938), where the term is used in our sense.

ON CERTAIN NON-LINEAR DIFFERENTIAL EQUATIONS OF THE SECOND ORDER

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The qualitative nature of the solutions of second order non-linear differential equations with a periodic forcing term is still largely an open matter. Here we shall state certain results we have succeeded in demonstrating for a class, C , of such equations of considerable importance in applied mathematics. Rather than define the Class C here, we indicate the nature of C by stating that included in C as a subclass are the equations

$$\ddot{x} + f(x) \dot{x} + g(x) = e(t)$$

where (1) $f(x)$, $g(x)$ and $e(t)$ are analytic functions for real values of x and t , respectively; (2) there exists some $a > 0$ such that $f(x) \geq a$; (3) $xg(x) > 0$ for large $|x|$, and $|g(x)| \rightarrow \infty$ as $|x| \rightarrow \infty$; (4) there exists some $b > 0$ such that for large $x > 0$, $g(\xi) > bg(x)$, $1/2x < \xi < x$; and (5) $e(t)$ has period L . (L we assume is the smallest period of $e(t)$.)

It is known that an equation of class C possesses at least one solution of period L .

Our results state that each equation of Class C falls into one of four exhaustive and mutually exclusive cases according to the qualitative behavior of its solutions as $t \rightarrow +\infty$. We now list for each case a necessary and sufficient condition to assure that an equation of Class C falls under that case and then give some further properties of the case.

Case I.—A necessary and sufficient condition for Case I is that the equation have more than one solution of period L .

Further properties of Case I:

- (a) There are an odd number, $2n + 1$, of solutions of period L of which $n + 1$ are stable and n are unstable as $t \rightarrow +\infty$.
- (b) All solutions, other than the n unstable ones of period L , tend to one of the $n + 1$ stable solutions of period L as $t \rightarrow +\infty$.

Case II.—A necessary and sufficient condition for Case II is that there exist at least one periodic solution of least period qL , q an integer > 1 . (Such a solution is called subharmonic.)

Further properties of Case II:

- (a) There exist no subharmonics with period distinct from qL .
- (b) The one solution of period L is stable if $q > 2$.
- (c) There are an even number of subharmonic solutions, half of which are stable and the other half unstable.

(d) All solutions, other than the several unstable subharmonic ones, tend to the stable subharmonic ones or to the solution of period L as $t \rightarrow +\infty$.

Case III.—A necessary and sufficient condition for Case III is that there exists only one periodic solution and that all other solutions tend to this one as $t \rightarrow +\infty$.

Case IV.—A necessary and sufficient condition for Case IV is that there exist only one periodic solution and that not all solutions tend to this one as $t \rightarrow +\infty$.

Further properties of Case IV:

- (a) The periodic solution is stable.
- (b) There are solutions which are of the type Birkhoff calls discontinuous recurrent.

We observe that any equation of Class C which has more than one periodic solution falls under Case I or Case II. Cases I, II and III are all known in practice. What conditions are required to exclude the possibility of Case IV, which we shall term the singular case, may be a hard question to answer. Case IV is intimately related to the singular case for motion on a torus.

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THE STRUCTURE AND FUNCTION OF THE GOLGI SYSTEM IN THE LIVING CELLS OF DEVELOPING MOLLUSCS

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Practically all of our knowledge of the Golgi apparatus has been built up through the study of fixed, osmic or silver, preparations, it not being generally realized that this important cytoplasmic inclusion can be revealed in many living cells by means of vital methylene blue staining. Reliance on the fixed preparations has created a great deal of confusion with regard to the structure of the Golgi bodies, because their nature is such that they only rarely lend themselves to faithful preservation by fixing fluids. In molluscs, these inclusions are of such large size that it has been possible to follow their behavior in intact embryos over periods of several days and considerable new information has been gathered regarding their structure and function.

Such observations reveal that the generalized Golgi body in molluscs is typically a more or less spherical, simple vesicle, possessing a chromophilic, gel-like, relatively lipoidal pellicle covering a relatively more protein, fluid, chromophobic core. The pellicle is thickened over one surface of the sphere forming a bowl-shaped structure which, through the microscope, looks like a crescent (Fig. 1 (A)). The relation between the thickened, bowl-shaped portion of the chromophile and the chromophobe is somewhat like the relation that exists between the "crescent" moon and the moon as a whole, except that in the Golgi body a thin layer of chromophilic material completely encloses the chromophobe. These generalized bodies are clearly endowed with the ability to keep the lipoidal and protein components segregated, hence the visible differentiation into chromophile and chromophobe. But they are the immediate descendants of homogeneous droplet-like forms where no such ability is manifest (Fig. 1 (B)). These droplets, in turn, are derived from smaller and presumably more solid granules which may be considered "reserve" forms (Fig. 1 (C)).

The enlargement of the granules to form droplets and the development of the droplets into larger, simple vesicles are due, with very little doubt, to the absorption of substances from the cytoplasm. This absorbing quality, together with the segregating tendency already referred to, constitute the two most fundamental physiological characteristics of the Golgi substance in these species.

In developing molluscs, the generalized Golgi bodies give rise, following gastrulation, to exceedingly active and highly specialized forms. By this period, quantities of the original yolk have been dissolved in the cytoplasm and although much has, no doubt, been oxidized, the Golgi elements

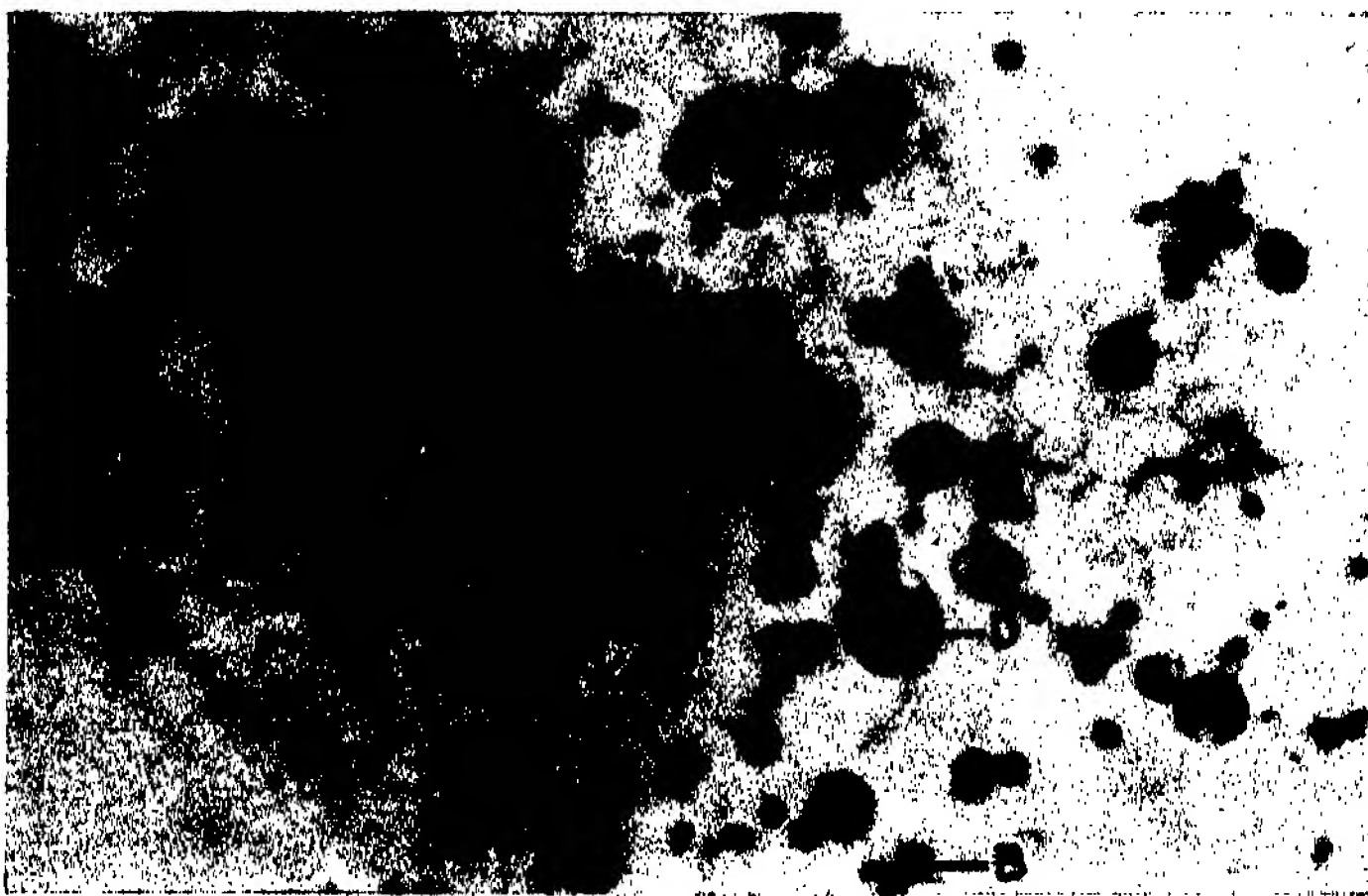


FIGURE 1

Living Golgi bodies seen in trochophore of the tectibranch *Navanax* ($\times 1000$). *A*, generalized form, showing "crescent"-like chromophile, and chromophobe; *B*, droplet; *C*, granule; *D*, compound vesicular forms, each showing several chromophobes.

absorb large amounts of the material and increase considerably in size, forming elaborate, compound vesicular bodies (Fig. 1 (*D*)). Within the chromophilic material, new vesicles continually make their appearance and, in the trochophore larva, small (1μ) fat droplets are continually discharged into the cytoplasm. In the pelecypod *Mytilus*, the absorption by the Golgi bodies of all of the ultramicroscopic, pigmented fat particles of the single-celled egg results in the freeing of the ground cytoplasm of pigment and the deposition of this pigment in the Golgi inclusions.

The discharge from each enlarged Golgi body of large ($4-6\mu$) protein spheres (the original chromophobes) is characteristic, in nudibranchs and tectibranchs, of the early veliger larva. In this process, the chromophilic portion of the structure withdraws from around the chromophobe and

condenses into a chromophilic, pycnotic mass, which can be shown to be rather solid. The pycnotic phase, however, is soon followed by a stage in which the mass becomes resolved into a number of spherical, relatively fluid, droplets. Each of these soon differentiates into a generalized vesicle as the segregating tendency is resumed and the cycle of the system begins anew.

The breaking up of the pycnotic Golgi body into droplets is the reproductive phase of the cycle and is the only method by means of which the Golgi elements increase in number during early development. In other words, there is no "dietyokinesis" accompanying every division of the cells, all multiplication being a mass division of the sort just described and always occurring after the assumption of a pycnotic condition following a period of marked synthetic activity. This also appears to be the situation during oögenesis where the synthetic period results in the deposition of large quantities of fat and protein yolk in the oöcyte. The multiplication period clearly increases the number of Golgi elements, thereby in tectibranchs providing a sufficient number for the new cells of the growing embryo. In the event that the embryo is not increasing in size during this period, as in *Mytilus*, this increase in number may be offset by a fusion phase preceding the active synthetic period. The fusion phase reduces the total number of Golgi bodies, but the original number is approximately restored during the multiplication period.

In the account just given, the internal chromophobe of each Golgi body is a potential protein or fat product which gradually becomes much more viscous as development proceeds. The elaboration of such products, in molluscs, occurs chiefly *within* the Golgi vesicle, but elaboration *outside* the vesicle is employed in *Mytilus* in the development of certain large oily yolk droplets. In this case, the product first makes its appearance within a group of small Golgi bodies, all of which appear to contribute to the single common yolk sphere. The mechanism involved in this process is not clear, but it is significant that elaboration of a product can take place merely through contact of the Golgi body with the ground cytoplasm.

In developing molluscs, the function of the Golgi system appears to be the continual mobilization of the fat and protein reserves within the cell. Why the embryo should continually dissolve its formed yolk inclusions, only to elaborate new ones is not immediately evident, but that the phenomenon is of fairly general occurrence in animals is indicated by the work of Schoenheimer¹ who finds that almost all of the proteins of the body are continually undergoing synthesis and breakdown. Should the Golgi system be found to be concerned in this protein turnover in higher animals, as it seems to be in the molluscs, it would prove to be a cytoplasmic constituent of greater metabolic significance than we have realized.

Summary.—For the first time, the Golgi system has been continuously followed in living, developing animal eggs. The multiplication of the Golgi elements has been observed and the rôle of the Golgi substance as an absorbing mechanism and protein and fat elaborating system is described. The active Golgi bodies are found to originate from minute, chromophilic “reserve” granules.

* Contribution from the Department of Biology, Brooklyn College, No. 45, and Contribution from The Scripps Institution of Oceanography, New Series No. 208.

¹ Schoenheimer, R., *The Dynamic Body Constituents*, Cambridge, Mass., 1942.

THE RELATION BETWEEN THE GOLGI APPARATUS AND “DROPLETS” IN THE CELL STAINABLE VITALLY WITH METHYLENE BLUE

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There has been considerable difference of opinion concerning the nature of certain “droplets” that become visible in cells when the tissue is stained with various basic vital dyes. One group of workers, notably Beams,¹ Ludford,² Chang³ and others, holds that these are “neoformations” resulting from the vital dye treatment and that they have no counterpart in the unaltered, living cell. Another group of investigators, notably Covell and Scott,⁴ Cowdry and Scott,⁵ Ma,⁶ Owens and Bensley⁷ and Parat,⁸ are of the opinion that these “dye droplets” represent the living Golgi material and that the classical Golgi apparatus of the fixed and osmicated cell results either from a precipitation of the osmic salt in and around these droplets, or from their running together and fusion as the result of fixation. In recent years, the tendency has swung in favor of the first contention, but crucial proof of neither of these views is at hand.

In my work, “droplets” of this kind have been revealed by means of supra- or intravital methylene blue staining of the tissues of many invertebrates, including the salivary gland cells of the larva of the midge *Chironomus*, and the smooth muscle, pancreas, liver and gall bladder of the frog and the smooth muscle, adipose tissue, pancreas, liver, kidney, thymus, bladder and pars distalis of the kitten. Usually, these “droplets” are opaque and show, in life, no internal structure and the reasons for their being considered “dye droplets” by so many workers are evident.

However, in all cases thus far examined, when living tissues containing

these "dye droplets" are treated with hypertonic salt solution, thus simulating the shrinkage that so frequently attends osmic acid fixation, the "droplets" rupture and form in each cell a group of fixtures that collectively so closely resemble the classical Golgi apparatus that there is little doubt but that the two are identical.

Experiments with hypertonic salt solution have shown that each "excretion droplet" is in reality a duplex structure, consisting of a rather thick,

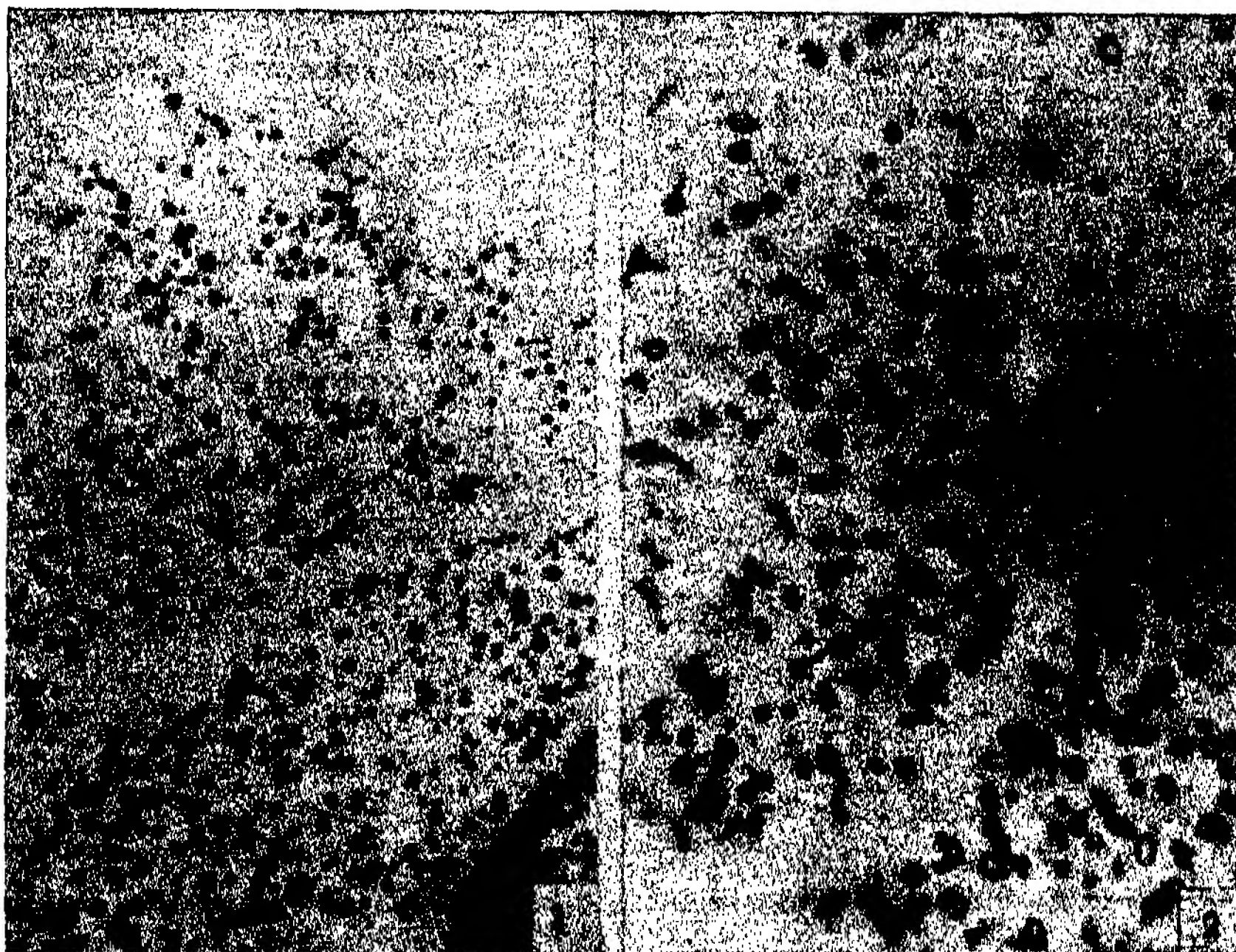


FIGURE 1

Living Golgi vesicles in the salivary gland of the larva of *Chironomus*, supravitaly stained with methylene blue ($\times 500$).

FIGURE 2

Same five minutes later after treatment with hypertonic salt solution ($\times 1000$). The vesicles have ruptured, producing the classical Golgi figures. Compare with figures of Beams and Goldsmith.⁹

chromophilic, gel-like pellicle and a fluid core, the latter corresponding to the chromophobe or osmiophobe of many invertebrate Golgi bodies. The fluid interior appears to be held under some slight pressure by the pellicle and the structure, far from being a mere droplet of dye is actually a simple turgid vesicle.

When these Golgi vesicles in the relatively inactive gland cells of *Chironomus* (Fig. 1), for example, are treated with hypertonic salt solution.

many chromophilic rings are immediately formed, each of which usually shows a localized thickening (Fig. 2). These vesicles, therefore, have an internal structure which no ordinary dye droplet could possibly be claimed to possess. Furthermore, the pellicle is revealed by these experiments to be much thicker than would be the case of a surface membrane that might conceivably be accredited to an excretion droplet. When relatively active gland cells are similarly treated, the fluid vesicle content squirts out into the cytoplasm forming chromophilic "streamers" which immediately solidify, or the entire vesicle may elongate forming a coarse thread. Under favorable circumstances, these threads and streamers, in the mammalian acinar cells of the pancreas, contact each other forming a chromophilic pseudoreticulum which bears a striking resemblance to the classical Golgi apparatus of the osmicated cell. This resemblance may be further increased by allowing the material to desiccate on the slide, thus simulating tissue dehydration. Frequently, both intact, and ruptured or elongated, vesicles may occur side by side in the same cell. This probably accounts for the widespread belief that since "dye droplets" or "vacuome (?)" (unruptured Golgi vesicles) and Golgi apparatus (ruptured or elongated Golgi vesicles) can be demonstrated in the same cell, the two are not identical. Indeed, this may be true in some tissues, but it is certainly not the case in those mentioned above.

When vitally stained amphibian smooth muscle cells are treated with hypertonic salt solution, the Golgi vesicles become flattened into discs which, upon desiccation, due to their soft gel-like consistency often fuse to form a longitudinal plate-like structure. It is probably in this manner that the classical lamellar form of the fixed Golgi apparatus in these cells is brought about by the fixing fluid. When vitally stained tissue is treated with hypertonic salt solution after a considerable delay following its removal from the animal, distortion of the vesicles is less marked, probably because on standing the vesicles undergo a considerable increase in viscosity.

These statements are not intended to imply that true excretion droplets never occur in vitally stained tissues, but it is perfectly clear that in the tissues thus far examined, the classical Golgi apparatus of the fixed cell is formed from chromophilic vesicles of the living cell that have frequently been considered "neoformations." It appears very probable that there is a correspondence between the "vacuome" and the Golgi system in far more instances than we have been accustomed to believe. At the same time, the vital methylene blue staining technique has revealed true Golgi puddingstone-like masses in invertebrate oöcytes and developing embryos which, in section, appear network-like.

Summary.—The living Golgi system in many invertebrate and vertebrate cells consists of a series of chromophilic vesicles, each with a rather

thick gel-like pellicle enclosing a fluid core. Upon treatment of the vitally stained tissue with hypertonic salt solution, thus simulating fixation shrinkage, these vesicles can be observed to collapse, discharge the fluid vesicle content or elongate forming a pseudoreticulum similar to that exhibited by the Golgi apparatus of the osmicated cell. Desiccation, superimposed upon the shrunken material, provides an even more striking picture of the classical Golgi apparatus.

* Contribution from the Department of Biology, Brooklyn College, No. 46, and Contribution from The Scripps Institution of Oceanography, New Series No. 209.

¹ Beams, H. W., *Anat. Rec.*, **45**, 137-162 (1930).

² Ludford, R. J., *Proc. Roy. Soc. London*, **B107**, 101-114 (1930).

³ Chang, H. C., *Anat. Rec.*, **62**, 95-104 (1935).

⁴ Covell, W. P., and Scott, G. H., *Ibid.*, **38**, 377-400 (1928).

⁵ Cowdry, E. V., and Scott, G. H., *Arch. de l'Institut Pasteur de Tunis* (1928).

⁶ Ma, W., *Chinese J. Physiol.*, **4**, 381-386 (1930).

⁷ Owens, H. B., and Bensley, R. R., *Am. J. Anat.*, **44**, 79-110 (1929).

⁸ Parat, M., *Arch. d' Mik. Anat.*, **24**, 73-357 (1928).

⁹ Beams, H. W., and Goldsmith, J. B., *J. Morph.*, **50**, 497-516 (1930).

MATTER, ELECTRICITY AND GRAVITATION IN FLAT SPACE-TIME

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In 1927 I presented two Notes¹ in which there was attempted a conceptual approach to the then new Schrödinger wave equation. This was done by taking matter to be a "perfect fluid," defined against the background of the curved space-time of Einstein's celebrated gravitational theory of 1916.

In February of last year I had the honor of presenting at Puebla and Tonantzintla, Mexico, before the Astrophysical Congress convening there, a new gravitational theory based on the same perfect fluid in the much simpler flat space-time characteristic of modern electromagnetic theory and special relativity. Already in 1912 Nordström had proposed a gravitational theory founded upon this type of space-time;² but his theory failed in that it did not account for the observed slight advance of the perihelion of Mercury beyond the amount indicated by the classical Newtonian theory.

My theory was obtained by laying down those demands which seemed most natural and elegant from the mathematical point of view.

In the present Note I wish to indicate in outline the modified account of matter, electricity and gravitation thus arrived at. The appropriate mathematical language is no longer that of tensors as in my two Notes of 1927, but is that of 4-vectors. It should be emphasized that, from the mathematical and philosophical point of view, the new theory is very simple.

I. NORMAL COÖRDINATES IN FLAT SPACE-TIME

Let ds denote the element of local time so that

$$ds^2 = dt^2 - dx^2 - dy^2 - dz^2$$

where dt and dx, dy, dz refer to the usual time and space coördinates in seconds and light-seconds, respectively. If now we replace t, x, y, z by $x^1 = t, x^2 = \sqrt{-1}x, x^3 = \sqrt{-1}y, x^4 = \sqrt{-1}z$, this formula takes the form

$$ds^2 = (dx^1)^2 + (dx^2)^2 + (dx^3)^2 + (dx^4)^2,$$

and the corresponding coördinates x^i are called normal coördinates. In such normal coördinates the language of 4-vectors becomes the same as that of 3-vectors in ordinary space. For this reason it is possible to use subscripts throughout rather than the subscripts and superscripts characteristic of tensor theory. Thus we have

$$ds^2 = dx_\alpha^2 \tag{1}$$

For brevity we shall use such normal coördinates almost exclusively.

II. THE PERFECT FLUID

By general consent the energy tensor of the homogeneous adiabatic fluid is written in essentially the form

$$T_{ij} = \rho u_i u_j - p \delta_{ij} \tag{2}$$

and, in terms of it, the equations of motion are written

$$\frac{\partial T_{ia}}{\partial x_a} = f_i \tag{3}$$

Here ρ and $p = f(\rho)$ designate, respectively, the density and pressure of the fluid, u_i is the velocity vector dx_i/ds , δ_{ij} is the usual Kronecker δ_{ij} , and f_i stands for the body force vector per unit of volume.

The perfect fluid is singled out by the further requirement that the disturbance velocity is to be that of light at all densities, with the corresponding equation of state,

$$p = \rho/2. \tag{4}$$

Only with this type of fluid can essential mathematical difficulties be avoided at collision of portions of the fluid.⁸ It is also assumed that there is an equilibrium density ρ_0 corresponding to a cosmic pressure $\rho_0/2$. The precise value of this density nowhere enters, and it would be equally possible to suppose that the equation of state has the form $p = (\rho - \rho_0)/2$ and that the pressure is 0 at the free boundaries in the customary manner.

We recall that the force vector f_i is necessarily orthogonal to the velocity vector u_i

$$f_\alpha u_\alpha = 0. \quad (5)$$

III. THE GENERAL HYPOTHESES ON THE FORCES

We propose now to make the following assumptions: (a) the force vector f_i is rational and integral in the velocity components, of not higher than the second degree; and (b) the coefficients are homogeneous and linear in the first partial derivatives of the corresponding potentials, namely, the atomic potential ψ which I introduced in 1927 (loc. cit.), the usual vector electromagnetic potential φ_i , and the symmetric gravitational tensor potential h_{ij} defined in the present Note. We shall furthermore suppose that (c) there are no degenerate quadratic terms in the velocities, i.e., no terms reducing to terms independent of u_i in view of the fundamental identity $u_\alpha^2 = 1$. These three types of potential seem appropriately designated, inasmuch as they refer primarily to matter, to electricity and to gravitation, respectively.

IV. THE ATOMIC POTENTIAL ψ

The components of the external forces acting on unit volume of the perfect fluid due to the atomic potential will, according to our general hypothesis above, be given by a vector of the form

$$c_{i\alpha} \frac{\partial \psi}{\partial x_\alpha}$$

of degree 0 in the velocities. Since $\partial\psi/\partial x_i$ is itself a vector it follows that $c_{i\alpha}$ must be a (numerical) tensor, and it is immediately obvious that it can only be a multiple of $\delta_{i\alpha}$. In fact if u_i and v_i are any two vectors, the associated rational invariants are their squared lengths u_α^2 , v_α^2 and the cosine of the angle between them, $(u_\alpha v_\alpha)/[(u_\alpha^2)(v_\alpha^2)]^{1/2}$. This fact shows that $c_{\alpha\beta} u_\alpha v_\beta$ must be $c \delta_{\alpha\beta} u_\alpha v_\beta$. Hence $c_{i\alpha} = c \delta_{i\alpha}$ as was stated, and the force vector under consideration must be $\partial\psi/\partial x_i$ up to a constant multiplier which may be absorbed into ψ .

But the condition (5) upon this component of f_i obviously yields

$$\frac{d\psi}{ds} = 0, \quad (A_1)$$

i.e., the atomic potential remains constant along the world line of any particle of the perfect fluid. It is assumed that ψ vanishes along the free boundaries and in empty space.

The formula for the corresponding atomic body force is, of course,

$$f_{A1} = \frac{\partial\psi}{\partial x_1}. \quad (A_2)$$

The primordially given atomic potential ψ supplies a useful mathematical instrument in the construction of a conceptual theory of matter and electricity. Thus in 1927 (loc. cit.) I showed how the atomic potential might be used to obtain an atomic frequency equation which closely resembled the celebrated wave equation of Schrödinger.

The model atom which I proposed was not positively unstable, although without rigidity. If it be required that the elementary constituents of matter, such as the proton, electron and neutron, *cannot* become locally concave this difficulty disappears (see my Oslo paper). We would have to suppose then that, in the moment when such concavity tends to be produced, there arises a tensional normal force at the surface just sufficient to prevent it. Under such a condition a closely packed set of elementary constituents would necessarily have polyhedral forms, and this property would seem to indicate a possibility of crystalline structure and rigidity. There would obviously be a tendency of such everywhere convex bodies to maintain a roughly spherical form under collisions and other strong disturbances.

V. THE ELECTROMAGNETIC POTENTIAL φ_1

As indicated above, the electromagnetic force vector f_{E1} , which arises from the terms linear in the velocities, is to be a vector of the form

$$c_{1\alpha\beta\lambda} \frac{\partial\varphi_\alpha}{\partial x_\beta} u_\lambda.$$

Since it is our intention to identify the potential φ_1 with the usual electromagnetic vector potential, we impose the condition

$$\frac{\partial}{\partial x_\alpha} \left(\frac{\partial\varphi_1}{\partial x_\alpha} - \frac{\partial\varphi_\alpha}{\partial x_1} \right) = -4\pi\sigma u_1, \quad (E_1)$$

where σ is the density of electricity. Now the only possible constituent terms in f_{E1} are to be obtained from

$$\frac{\partial \varphi_p}{\partial x_q} u_r$$

by a single contraction of indices and choice of a subscript i , in the three possible ways:

$$\frac{\partial \varphi_i}{\partial x_\alpha} u_\alpha, \quad \frac{\partial \varphi_\alpha}{\partial x_i} u_\alpha, \quad \frac{\partial \varphi_\alpha}{\partial x_\alpha} u_i.$$

Consequently the force vector in question is of the form

$$a \frac{\partial \varphi_i}{\partial x_\alpha} u_\alpha + b \frac{\partial \varphi_\alpha}{\partial x_i} u_\alpha + c \frac{\partial \varphi_\alpha}{\partial x_\alpha} u_i.$$

But by the general requirement (5) this has to vanish for $i = 1$ when $u_1 = 1$, $u_2 = u_3 = u_4 = 0$, i.e.,

$$(a + b) \frac{\partial \varphi_1}{\partial x_1} + c \frac{\partial \varphi_\alpha}{\partial x_\alpha} \equiv 0.$$

Since there is no necessary relation between $\partial \varphi_1 / \partial x_1$ and $\partial \varphi_\alpha / \partial x_\alpha$, we infer that $a + b = c = 0$. Hence this electromagnetic force vector is essentially

$$f_{Ei} = \sigma \left(\frac{\partial \varphi_i}{\partial x_\alpha} - \frac{\partial \varphi_\alpha}{\partial x_i} \right) u_\alpha \quad (E_2)$$

since the ponderomotive force is proportional to the electrical density σ .

VI. THE GRAVITATIONAL POTENTIAL h_{ij}

By analogy with the Poisson equation in classical gravitational theory, we shall assume that a similar equation holds in each separate component of the symmetric energy tensor T_{ij} and the corresponding component h_{ij} of the symmetric gravitational potential h_{ij} , namely,

$$\frac{\partial^2 h_{ij}}{\partial x_\alpha^2} = 8\pi T_{ij}, \quad (G_1)$$

where the multiplier 8π is selected for reasons of convenience. This condition (G_1) is evidently the simplest analogous equation from the formal point of view.

According to our initial hypothesis the types of terms which may enter in the corresponding gravitational force vector f_{Gi} , arising from the quadratic terms in the velocities, are derived from

$$\frac{\partial h_{pq}}{\partial x_r} u_i u_j$$

by a double contraction of indices and choice of the index i . This yields six possible types:

$$\frac{\partial h_{i\alpha}}{\partial x_\beta} u_\alpha u_\beta, \quad \frac{\partial h_{\alpha\beta}}{\partial x_i} u_\alpha u_\beta, \quad \frac{\partial h_{\alpha\beta}}{\partial x_\alpha} u_\beta u_i, \\ \frac{\partial h_{\alpha\alpha}}{\partial x_\beta} u_\beta u_i, \quad \frac{\partial h_{i\alpha}}{\partial x_\alpha} u_\beta^2, \quad \frac{\partial h_{\alpha\alpha}}{\partial x_i} u_\beta^2.$$

But the last two types reduce respectively to $\partial h_{i\alpha}/\partial x_\alpha$ and $\partial h_{\alpha\alpha}/\partial x_i$ since $u_\beta^2 = 1$, and so are of the degenerate type excluded by the hypothesis (c). Thus the most general available gravitational force vector is of the form

$$a \frac{\partial h_{i\alpha}}{\partial x_\beta} u_\alpha u_\beta + b \frac{\partial h_{\alpha\beta}}{\partial x_i} u_\alpha u_\beta + c \frac{\partial h_{\alpha\beta}}{\partial x_\alpha} u_\beta u_i + d \frac{\partial h_{\alpha\alpha}}{\partial x_\beta} u_\beta u_i$$

But by (5) this must vanish for $i = 1$ if $u_1 = 1, u_2 = u_3 = u_4 = 0$, so that

$$(a + b) \frac{\partial h_{11}}{\partial x_1} + c \frac{\partial h_{\alpha 1}}{\partial x_\alpha} + d \frac{\partial h_{\alpha\alpha}}{\partial x_1} = 0.$$

Since the quantities $\partial h_{11}/\partial x_1$, $\partial h_{\alpha 1}/\partial x_\alpha$ and $\partial h_{\alpha\alpha}/\partial x_1$ are independent of one another we must have $a + b = c = d = 0$. This yields for the gravitational force vector essentially the following expression

$$f_{\sigma i} = \rho \left(\frac{\partial h_{i\alpha}}{\partial x_\beta} - \frac{\partial h_{\alpha\beta}}{\partial x_i} \right) u_\alpha u_\beta, \quad (G_2)$$

where the factor ρ is introduced since the gravitational force is proportional to the density ρ .

VII. THE COMPLETE THEORY

We have then an energy tensor T_{ij} given by (2) with $p = \rho/2$. The equations of motion are given by (3) where

$$f_i = f_{Ai} + f_{Ki} + f_{Gi}.$$

The three terms on the right are defined by (A_2) , (E_2) , (G_2) , and are of degrees 0, 1, 2 in the velocities. Furthermore the equations (A_1) , (E_1) , (G_1) determine, respectively, the atomic, electromagnetic and gravitational potentials involved. Thus we have 21 dependent variables, $\rho, \sigma, u_i, \psi, \varphi_i, h_{ij}$, and we have 20 equations in these variables, namely, the 4 equations of motion (2), the single equation (A_1) , the 4 equations (E_1) , the 10 equations (G_1) and in addition the relation $u_\alpha^2 = 1$. This is as it should be since the electromagnetic potential φ_i is only determined up to the gradient of an arbitrary function, $\partial V/\partial x_i$. It is to be remembered that in empty space we take the atomic potential ψ and the energy tensor T_{ij} to vanish.

This system of equations is complete and consistent as a mathematical embodiment of matter, electricity and gravitation. The corresponding system in the generalized theory of Einstein is incomplete to the extent that the equation of state of the homogeneous adiabatic fluid is not specified, and it is inconsistent in that when two portions of the fluid collide, the equation of motion may break down.

VIII. RÉSUMÉ OF THE NEW GRAVITATIONAL THEORY

Let us now disengage as far as possible the new gravitational theory and consider to what extent its predictions are in agreement with the known facts.

Matter is supposed to be either that special, mathematically satisfactory, homogeneous adiabatic fluid for which $p = \rho/2$ or, presumably, any form of matter in which the disturbance velocity is that of light under all circumstances.

We take T_{ij} to designate the energy tensor of matter and suppose that the equations of motion in the absence of a gravitational field (i.e., when only a small quantity of matter is present) may be written in the usual form:

$$\frac{\partial T_{ia}}{\partial x_a} = \bar{f}_i,$$

where \bar{f}_i is a suitable force vector. It is then assumed that in the case of a gravitational field we may write the force vector f_i in the form

$$f_i = \bar{f}_i + f_{gi},$$

where f_{gi} designates the gravitational force vector.⁴

We assume further that there is an associated symmetric gravitational tensor potential h_{ij} such that a Poisson equation (G_1) holds in each component of h_{ij} relative to T_{ij} . In empty space T_{ij} is taken to vanish.

Under these circumstances f_{gi} cannot be independent of the velocities since a proper force vector must be orthogonal to the velocity vector. Moreover since gravitational theory is reversible in time (in contradistinction to electromagnetic theory), and since in the classical theory the gravitational force components are given by the components of the gradient of the gravitational potential, we assume that the gravitational force vector is homogeneous and quadratic in the velocity components, and homogeneous and linear in the first derivatives of the components of the gravitational potential, and furthermore that none of its components are degenerate, i.e., involve a factor $u_a^2 = 1$.

In this way we obtain for the gravitational force vector f_{gi} the unique expression (G_2). Thus our complete system of equations is

$$\frac{\partial T_{ia}}{\partial x_a} = \bar{f}_i + f_{oi}, \quad \frac{\partial^2 h_{ij}}{\partial x_a^2} = 8\pi T_{ij},$$

where we regard f_{oi} and T_{ij} as replaced by their explicit expressions.

It is our intention in conclusion to indicate why this simple theory of gravitation set in the framework of flat (i.e., classical electromagnetic) space-time is in agreement with the observed facts.

IX. GRAVITATION IN THE QUASI-STATIONARY STATE

Let us suppose first that the portions of the perfect fluid are moving at small velocities relative to some frame of reference so that we have approximately $u_1 = 1, u_2 = u_3 = u_4 = 0$. In this case we find in the coördinates $x^1 = t, x^2 = x, x^3 = y, x^4 = z$,

$$\delta h_{ij} = -4\pi\rho\delta_{ij} \quad \left(\Delta = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2} \right).$$

approximately so that h_{ij} is negligible for $i \neq j$ while h_{ii} for $i = 1, 2, 3, 4$ reduce to the ordinary gravitational potential g . The gravitational force vector per unit of mass then reduces to the gradient of g . Thus the theory is in first order agreement with the Newtonian theory.

X. THE CENTRALLY SYMMETRIC STATE

Suppose next that we have a sphere of the perfect fluid at rest with its center at $(x, y, z) = (0, 0, 0)$. Of course T_{ij} and h_{ij} will then be independent of the time t , and our Poisson tensor equation reduces to the equation written above in the same coördinates so that we obtain for all i and j the exact equation outside of the sphere

$$h_{ij} = \frac{m}{r} \delta_{ij} \quad (r, \text{radial distance})$$

where m is the mass of the fluid sphere.

Thus the three *exact* equations of motion for a particle at (x, y, z) attracted by the sphere are found to be of the type

$$x'' = -\frac{mx}{r^3} - \frac{2mx}{r^3} (x'^2 + y'^2 + z'^2) + \frac{mx'r'}{r^3},$$

where the accent ' indicates differentiation as to s . It is to be observed that the first terms on the right yields the dominant Newtonian force components, the other two small terms being relativistic in origin.

Now there is no essential restriction in assuming that the initial plane of the motion through $(0, 0, 0)$ is the z -plane, whence we can at once conclude that $z = 0$ for all time. Hence we have only to solve the first two equations

with $z = z' = 0$, $r = \sqrt{x^2 + y^2}$. This is a readily integrable pair of equations with x' , y' and y , $-x$ as two pairs of integrating factors.

The differential equation of the path of the particle is seen to be

$$h^2 \left(\left(\frac{du}{d\theta} \right)^2 + u^2 \right) = e^{2(mu + C)} (e^{2(mu + C)} - 1),$$

where $u = 1/r$ and θ is the longitude; h and C are arbitrary constants of integration. This may be integrated by an obvious quadrature.

It is thus readily established that the resultant formulas for the advance of perihelion of the particle (x, y, z) and the deviation of a ray of light (thought of as the path of a photon) in the gravitational field of the central sphere of matter are the same in their principal parts as in the Einstein theory. Furthermore the formula for the spectral shift toward the red is also in essential agreement with that theory. The exact expressions are, however, different.⁵

Thus the simple theory of gravitation here outlined seems well adapted to explain the known physical facts. Like the Einstein theory, it has the advantage of involving no arbitrary constants whatsoever. However, it is essentially different in that it presupposes a framework of flat space-time instead of space-time curved by matter, and a basic form of matter in which the disturbance velocity is that of light.

¹ "A Theory of Matter and Electricity," "The Hydrogen Atom and the Balmer Formula," these PROCEEDINGS, vol. 13, 1927. See also my article, "The Foundations of Quantum Mechanics," in the *Proceedings of the International Mathematical Congress at Oslo*, 1 (1936).

² "Relativitätsprinzip und Gravitation," *Physikalische Zeitschrift*, 13 (1912).

³ In a paper about to appear in the *Revista de Ciencias de Lima*, entitled "Sobre el Fluido Perfecto," I have shown by direct integration that at least in two-dimensional space-time, such difficulties do not arise with the perfect fluid.

⁴ It is a mistake to believe that the Einstein theory of gravitation does not similarly *superimpose* a gravitational force upon the other forces. It is only the mechanism of the superimposition which is different in the Einstein theory. In fact any physical theory *without gravitation in flat space-time* becomes one *with gravitation in curved space-time* when ordinary derivatives are replaced systematically by the covariant derivatives of the tensor calculus.

⁵ The details of the new gravitational theory will appear in my article "El concepto de tiempo y la gravitación" in the *Proceedings of the Astrophysical Congress* held at Puebla and Tonantzintla, Mexico, in February, 1942.

GROUPS OF TRANSFORMATIONS OF THE NON-INVARIANT SUBGROUPS

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All the non-invariant subgroups of a given group G are transformed under G according to a permutation group K whose degree is equal to the number of these subgroups, but not every permutation group of an arbitrary degree n is the group of transformations of the subgroups of some group. In particular, it will be proved in what follows that no imprimitive permutation group is the group of the transformations of all the non-invariant subgroups of some possible group and all the possible primitive permutation groups which are the groups of the transformations of the non-invariant subgroups of some group under this group will be determined. It therefore will remain only to determine some of the possible intransitive permutation groups which can be the groups of the transformations under a group of all its non-invariant subgroups of G .

The operators of G which correspond to the identity of K are composed of all the operators of G which transform each of its subgroups into itself and they constitute a subgroup of G which has been called the *norm* of G .¹ To emphasize the fact that they constitute an important special characteristic subgroup of G we shall call this subgroup the *character subgroup* of G . A necessary and sufficient condition that the character subgroup of G is G itself is that G is either abelian or hamiltonian. In all other cases the character subgroup of G is a proper subgroup of G whose index under G is at least as large as 2, and when it is of this index it cannot be hamiltonian and it must therefore be abelian.

To prove this fact it may first be noted that whenever the character subgroup of G is of prime index under G it involves all of the non-invariant subgroups of G . In particular, it involves all the operators of prime order contained in G . If the character subgroup of G is of index 2 under G it must therefore contain all the operators of order 2 which appear in G . There must be operators in G whose order is a power of 2 and which are not contained in the character subgroup of G . If this subgroup were non-abelian and would have an order which is a power of 2 it would also contain all the operators of order 4 which appear in G . If this were not the case an operator of order 4 which would not be found in the character subgroup of G would generate an invariant subgroup of G but its square could not be the square of the operators of order 4 found in the character subgroup of G since this subgroup contains all the operators of order 2 which appear in G .

It therefore results that the operators of order 4 which would not appear

in the character subgroup of G would have different squares. As this is clearly impossible and since not all of the operators of G which do not appear in its character subgroup could be of order 8 as a result of the properties of the possible groups of order 16 it has been proved that *whenever the character subgroup of G is of index 2 it must be an abelian subgroup of G .* When the character subgroup of G is of a larger prime index under G it is not necessarily abelian as results directly from the fact that a direct product of the quaternion group and a group which involves a prime number of conjugate subgroups, while all its other subgroups are invariant, contains a non-abelian character subgroup of prime index, where this prime is an arbitrary odd prime number.

It is known that a regular group of any prime order is the group of the transformations of all the non-invariant subgroups of some group.² We proceed to prove that no regular group of composite order can have this property. This will be done by showing that we arrive at a contradiction by assuming that a regular permutation group of composite order is the group of the transformations of all the non-invariant subgroups of some group. Two facts which should be kept in mind in this proof are that all the non-invariant subgroups contained in G would be conjugate under G and that all these non-invariant subgroups would appear among the operators of G which would correspond to the identity of K .

To simplify this proof we shall first consider the case when this regular group is of even order. To a permutation group of order 2 in K there would correspond in G a group whose character subgroup would be of index 2 under the group and hence this character subgroup would be abelian. Hence this group would have a commutator subgroup of order 2 and all of its operators which do not appear in the given character subgroup would generate this commutator subgroup since each of these operators would generate an invariant subgroup of G . If K would involve an operator of order 4 it may be assumed without loss of generality that this operator would generate the given permutation of order 2 contained in K . This clearly leads to a contradiction since the operators of G which correspond to the given permutation group of order 2 in K would transform all the non-invariant subgroups of G among themselves. This would also be true if K would involve a non-cyclic subgroup of order 4.

The fact to be emphasized in this connection is that the given subgroup of order 2 in K would be invariant under K since the corresponding subgroup of G would be invariant under G . If the order of K is not divisible by 4 then K must contain a permutation of odd prime order which is commutative with the given permutation of order 2. To this permutation there would correspond in G an operator which would give rise to a commutator of odd order. As this is impossible we have arrived at a contradiction by assuming that the order of K is an even number. If K would

have an odd order it is easy to see that the character subgroup of G could not have an order which is a power of 2 and hence that this subgroup would again be abelian. It therefore results that K cannot be a regular group of composite order.

If K would be an imprimitive non-regular group its subgroup composed of all its permutations which omit a given letter could not be maximal and hence G would contain non-invariant subgroups of different orders. These could not be conjugate and hence a proof of the fact that a *non-regular imprimitive group cannot be the group of the transformations of all the non-invariant subgroups of some group under this group* is a direct consequence of the fact that in such a group the permutations which omit a given letter form a non-invariant proper subgroup which is contained in a larger non-invariant proper subgroup of the imprimitive group.

When K is primitive and non-regular its subgroups composed of all its permutations which omit a given letter omit only one letter and hence the number of these subgroups in K is the same as its degree. It therefore results that these subgroups are of prime order and hence that K involves a regular subgroup as an invariant subgroup. Moreover, this subgroup must be of prime order since the number of the non-invariant subgroups of K cannot exceed its degree. It therefore results that *if a non-regular primitive group is the group of the transformations of all the non-invariant subgroups of some group under this group its order is the product of two distinct prime numbers which are such that the larger of these two prime numbers diminished by one is divisible by the smaller*. This condition is obviously both necessary and sufficient.

The intransitive permutation group of prime order and of a degree which is an arbitrary multiple of this order is the group of the transformations of all the non-invariant subgroups contained in some group. In particular, it is clearly the group of the transformations under the group of all the non-invariant subgroups of the group obtained by forming the direct product of a prime power cyclic group and groups which satisfy the condition that each contains a number of conjugate subgroups which is equal to this prime number but contains no other non-invariant subgroup. It was noted above that such groups exist for every prime number and that when the prime is odd the group itself may be the group of the transformations of its conjugate subgroups, as is the case, for instance, when the group is the symmetric group of order 6.

¹ Cf. Baer, R., *Amer. Jour. Math.*, 61, 700 (1939).

² These PROCEEDINGS, 29, 106 (1943).

ON THE UNIFORM CONVERGENCE OF THE SOLUTIONS OF THE NAVIER-STOKES EQUATIONS

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Let \mathfrak{R} be a fixed region of space completely filled by a viscous fluid. We assume \mathfrak{R} to be finite and to be bounded by one or more surfaces each of which may be at rest or in motion. In the case of a fluid occupying an infinite region, for example, a hypothetical infinite pipe, \mathfrak{R} , may be taken to be the periodicity cell which it is customary to introduce for convenience in such discussions.

Denote by \bar{u}^α ($\alpha = 1, 2, 3$) and \bar{p} the contravariant velocity components and the pressure function of a particular (stationary or non-stationary) fluid motion in \mathfrak{R} . Similarly u^α and p will represent the corresponding functions for any other motion of the fluid in \mathfrak{R} . It will be assumed that the viscosity μ and density ρ of the fluid are constant and that all motions satisfy the Navier-Stokes equations and the equation of continuity. The quantities $\xi^\alpha = u^\alpha - \bar{u}^\alpha$ and $\xi^4 = p - \bar{p}$ are the components of an invariant ξ called the *disturbance*. We shall derive general conditions under which all such disturbances converge uniformly to zero in \mathfrak{R} whereupon the above particular motion \bar{u}^α, \bar{p} will be said to be stable relative to the class of motions u^α, p (or disturbances ξ) in question.

Consider the following inequality

$$g_{\alpha\beta} g^{\sigma\tau} (\xi_{,\sigma}^\alpha + g_{\gamma\sigma} \xi^\alpha f^\gamma) (\xi_{,\tau}^\beta + g_{\delta\tau} \xi^\beta f^\delta) \geq 0 \quad (1)$$

valid in any Riemann space (positive definite form). In (1) the quantities $\xi_{,\sigma}^\alpha$ are the components of the covariant derivative of the spatial part of the disturbance ξ and the functions f^α are assumed to be continuous and have continuous first partial derivatives in the region \mathfrak{R} . Expanding the left member of (1), integrating over \mathfrak{R} , making use of Green's theorem and the boundary condition ($\xi^\alpha = 0$ on the boundary of \mathfrak{R}) we find

$$\int g_{\alpha\beta} g^{\sigma\tau} \xi_{,\sigma}^\alpha \xi_{,\tau}^\beta dV \geq \int (f_{,\sigma}^\sigma - g_{\gamma\delta} f^\gamma f^\delta) g_{\alpha\beta} \xi^\alpha \xi^\beta dV. \quad (2)$$

In the case of periodic disturbances for which \mathfrak{R} is the periodicity cell the condition of spatial periodicity combined with the above boundary condition over the actual surfaces limiting \mathfrak{R} suffices for the derivation of this inequality. From (2) and the energy equation for the disturbance (derived from the Navier-Stokes equations) we obtain the inequality

$$\frac{dK}{dt} + \int [\mu(f_{,\sigma}^{\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta}) g_{\alpha\beta} + \rho D_{\alpha\beta}] \xi^{\alpha} \xi^{\beta} dV \leq 0, \quad (3)$$

in which the $D_{\alpha\beta}$ are the components of the symmetric deformation tensor of the motion \bar{u}^{α} , \bar{p} and K is the kinetic energy of the disturbance.

Now the functions f^{α} can be chosen so that the form

$$(f_{,\sigma}^{\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta}) g_{\alpha\beta} \xi^{\alpha} \xi^{\beta} \quad (4)$$

is positive definite in the closure $\bar{\mathfrak{R}}$ of \mathfrak{R} , the condition for this being merely the existence of the condition $f_{,\sigma}^{\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta} > 0$ over $\bar{\mathfrak{R}}$. It is easily seen that this condition can be satisfied. For example if we introduce rectangular Cartesian coördinates x, y, z with origin in \mathfrak{R} and take $f^1 = F(x)$, $f^2 = f^3 = 0$ the condition will be met if $dF/dx - F^2 = c^2$ where c is a constant different from zero. Integration of this equation gives $F = c \tan cx$ and hence F will be continuous and differentiable in $\bar{\mathfrak{R}}$ if c is sufficiently small. It follows that (4) can be made positive definite in $\bar{\mathfrak{R}}$ as above stated. Hence if the deformation tensor D is sufficiently small (at all times) the integrand in (3) will be positive definite. Under this condition it follows from (3) that K , the kinetic energy of the disturbance, will continually decrease and from the physical standpoint one must expect that this will result in the gradual "damping out" of the disturbance. A strict mathematical proof of the fact that the disturbance converges uniformly to zero can be made on the basis of certain assumptions concerning the continuity and finiteness of the disturbance and certain of its derivatives and application of Ascoli's theorem on the convergence of sequences. The details of proof are essentially the same as those of a proof given in a previous paper: "Qualitative Analysis of the Flow of Fluids in Pipes," *Am. Jour. of Math.*, LXIV, 754-767 (1942). Understanding that these conditions are met we are thus led to the following result. *Any motion \bar{u}^{α} , \bar{p} is stable in the region \mathfrak{R} relative to arbitrary finite disturbances provided that its deformation tensor D is sufficiently small.* It follows in particular that if the deformation tensor vanishes the motion is always stable.

As a specific example consider the stability of Poiseuille motion in a circular pipe relative to arbitrary spatially periodic disturbances. For this motion the deformation tensor has the components $D_{12} = D_{21} = -w_0 r/a^2$ where we have referred the motion to cylindrical coördinates r, ϕ, z (the z -axis coinciding with the axis of the pipe) and where a is the radius of the pipe and w_0 the velocity of the fluid along the axis. The other components of the deformation tensor vanish. The conditions for the integrand in (3) to be positive definite can be expressed in the usual manner in terms of the elements of the symmetric matrix

$$\left\| \begin{array}{ccc} \mu(f_{,\sigma}^{\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta}) & 0 & -\frac{\rho w_0 r}{a^2} \\ 0 & \mu(f_{,\sigma}^{\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta}) & 0 \\ -\frac{\rho w_0 r}{a^2} & 0 & \mu(f_{,\sigma}^{\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta}) \end{array} \right\|$$

On observation we see that these conditions reduce to the single inequality

$$f_{,\sigma}^{\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta} > \frac{Rr}{a^3}, \quad (5)$$

where $R = w_0 a / \nu$ is the Reynolds number, the quantity ν being the kinematical coefficient of viscosity. To treat the condition (5) let us make the substitution $f_{,\sigma} = -\psi_{,\sigma} / \psi$. The inequality (5) then becomes

$$\Delta\psi + \frac{Rr}{a^3} \psi < 0, \quad (\psi > 0), \quad (6)$$

where the condition $\psi > 0$ is necessitated by the above requirement that the functions f_{α} shall be continuous and differentiable in \mathfrak{R} . We can suppose the inequality sign in (6) to be replaced by the equality sign as a limiting case. Or if we like we can think of this equation as involving a slightly increased value of R in which case it will be equivalent for our purpose to the strict inequality (6). With this in mind we now suppose that ψ is dependent on r alone and make the change of independent variable $x = r^{3/2}$ whereupon the condition (6) becomes

$$\frac{d^2\psi}{dx^2} + \frac{1}{x} \psi + \frac{4R}{9a^3} \psi = 0, \quad (\psi > 0). \quad (7)$$

A solution of (7) is given by the Bessel function J_0 . In fact we have

$$\psi = J_0 \left(\sqrt{\frac{4R}{9a^3}} x \right) = J_0 \left(\sqrt{\frac{4Rr^3}{9a^3}} \right).$$

The requirement $\psi > 0$ in \mathfrak{R} means that the argument of the above function J_0 shall not exceed the value of the first root of this function in the interval $0 \leq r < a$. This gives $\sqrt{4R/9} \leq 2.4$ or $R \leq 13$ (in round numbers).

There seem to be two distinct mathematical problems concerning the above Reynolds number R according to experimental evidence. First, it is well known that large disturbances are damped out when R does not exceed a value around 1000. However, recent experiments have shown that even for very large values of R disturbances are likewise damped out provided they are sufficiently small.¹ There appears in fact to be no upper limit to the value of R beyond which small disturbances will not converge to zero. To account for this one may have recourse to the so-called linearized equa-

tions of the disturbance which can be justified strictly only under the assumption of arbitrarily small disturbances and this theory should thus lead to the stability of Poiseuille motion relative to such disturbances for any value of the Reynolds number. In spite of this, stability has been proved only for comparatively small values of R and even then only under the additional assumption of axial symmetry. Thus Orr² has arrived at the value $R < 180$ for stability of Poiseuille motion relative to infinitely small axial symmetric disturbances. The problem of showing the existence of a critical value of R at approximately 1000 is a problem involving finite disturbances and the general theorem of this paper has led only to the insufficient value $R \leq 13$ for the stability of Poiseuille motion relative to these disturbances. As far as I am aware there is no previous discussion in the literature of the stability of Poiseuille motion relative to finite disturbances on the basis of the Navier-Stokes equations.

¹ See Prandtl, L., and Tietjens, O. G., *Applied Hydro- and Aeromechanics*, McGraw-Hill, 32-35 (1934) for a more complete discussion and references to the literature.

² Orr, W. McF., "The Stability or Instability of the Steady Motions of a Liquid. Part II: A Viscous Liquid," *Proceedings of the Royal Irish Academy*, (A), 27, 135 (1907).

For a comprehensive discussion of stability relative to small displacements see Synge, J. L., "Hydrodynamical Stability," *Am. Math. Soc. Semicentennial Publications*, II: Addresses, 227-269 (1938).

RELATIONSHIP BETWEEN PARTICLE SIZE AND EFFICIENCY OF PALLADIUM-POLYVINYL ALCOHOL (Pd-PVA) CATALYSTS*

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Spectrophotometric measurements¹ carried out on PVA and silver have proved that freezing is a suitable means for obtaining reproducible changes of the size of particles of colloids. Continued investigations in this laboratory on colloidal noble metals, such as palladium, platinum and rhodium, in conjunction with synthetic high polymers and vanadium resulted in preparing supported catalysts, which have shown themselves to be extremely efficient not only from a kinetic² but also from a chemical³ standpoint.

It was shown in a recent communication⁴ that oriented nickel films with the same available surface as unoriented films had five times the activity as measured by ethylene hydrogenation. An attempt was made, accordingly, to obtain insight into the causes for increased activity of Pd-

PVA catalysts by coördinating ultracentrifugation of such solutions with measurements of hydrogen consumption, the rate of hydrogenation of nitrobenzene^{4a} and determination of the size and shape of catalyst particles through electron micrographs.

Measurements of Gas Consumption.—First, it was attempted to determine whether any difference existed between the surface area or particle size of Pd in two types of colloidal supports. PVA was chosen as the representative member of the synthetic polymer series, and gum arabic (GA) as the representative of the naturally occurring type of colloid. The amount of gas adsorbed served as a means of measuring the surface area: for the surface area can be measured by the volume of gas adsorbed. Thus a difference in the volume of gas adsorbed by the Pd supported by PVA and by Pd supported by GA would serve as a means of distinguishing between the size of Pd particles in each medium.

Apparatus.—A thermostat was set at 30°C. \pm 0.01. Special flasks were designed to fit constant pressure manometers, whereby the amounts of gas adsorbed were measured. The volume of the vessels which were cylindrical in shape was approximately 300 cc. Mercury served as the confining liquid in the manometers and dibutylphthalate was used in the supplementary capillary manometers.

Two hundred and fifty cubic centimeter samples of 1% PVA solution containing 50 mg. of Pd were prepared from 125 cc. of a 2% PVA solution, 120 cc. of distilled water and 5 cc. of a PdCl₂ solution containing 10 mg. of Pd per cubic centimeter, i.e., 1% with regard to Pd. As a blank, a 1% PVA solution was made up from 125 cc. of 2% PVA and 125 cc. of distilled water.

These solutions were immersed in the thermostat overnight to bring them to constant temperature. The next day, the special flasks, immersed in the thermostat and attached to the manometers, were evacuated by aspirator, filled with hydrogen and then flushed with H₂ seven times.

The hydrogen adsorption on the colloidal palladium was the difference between the PVA-Pd solution and the blank. The blank reading was added if the volume in the blank solution increased; subtracted if the volume in the blank solution decreased.

The total gas consumption figure thus obtained was diminished by the amount of hydrogen which is necessary to reduce the PdCl₂ (20 mg. Pd) to metallic Pd. Thus, the absolute adsorption is the total gas consumption figure minus 4.20 cc. For comparison purposes, however, the total gas consumption figure was used.

The results obtained with PVA-Pd and GA-Pd, in which the colloids were present in a concentration of 1% and 0.5%, respectively, are presented in table 1.

TABLE 1

HYDROGEN CONSUMPTION BY GA-Pd AND PVA-Pd SOLUTIONS (100 Cc.) WHICH HAVE STOOD IN THERMOSTAT 16-20 HOURS

COLLOIDAL CATALYST	CC. OF H ₂ ADSORBED	
	0.5%	1%
GA-Pd	8.30 \pm 0.20	8.30 \pm 0.20
PVA-Pd	6.50 \pm 0.20	3.90 \pm 0.20

Two observations are of interest. First, a greater hydrogen consumption indicating a greater surface, is possessed by the inferior GA-Pd catalyst; further, an increase in consumption is shown by the PVA series when the concentration is decreased to 0.5%, whereas the GA series shows identical hydrogen consumption for both concentrations.

It had been noticed that the reddish colloidal solution containing PVA and PdCl₂ had turned to a blackish solution on standing for several hours (without hydrogen) in the thermostat. The GA-PdCl₂ solution did not exhibit this property but retained its light yellow translucent color. To circumvent this darkening, the PVA solutions were made up, let stand in the thermostat overnight and then on the next morning, immediately before introduction into the measuring vessels, made up to volume of 250 cc. by the addition of 5 cc. of the PdCl₂ solution. The same was done with the GA catalyst. By this treatment, no darkening occurred in the PVA solutions.

With the GA-Pd solutions, no change in hydrogen adsorption was noted. However, striking results were obtained with the PVA catalysts. PVA (1%)-Pd showed a total hydrogen consumption of 8.05 cc. \pm 0.20; while a PVA (0.5%)-Pd showed a gas consumption of 8.25 cc. \pm 0.20. The values given here as well as all other figures previously recorded, were the mean values of six measurements. It was apparent, then, that there was no significant difference between the adsorption of 1% and 0.5% PVA when the solutions were made up immediately before placement in the measuring vessels. These results are recorded in table 2. In table 3, all values are converted from "Total Gas Consumption" to "Absolute Adsorption," i.e., the amount of hydrogen required for reduction of PdCl₂ is subtracted.

TABLE 2

H₂ CONSUMPTION BY "FRESHLY PREPARED" PVA-Pd AND GA-Pd CATALYSTS. 100 CC. OF SOLUTION CONTAINING 20 MG. OF Pd ARE USED IN ALL CASES

CATALYST SOLUTION	CC. OF H ₂ ADSORBED	
	0.5%	1%
GA-Pd	8.30 \pm 0.20	8.30 \pm 0.20
PVA-Pd	8.25 \pm 0.20	8.05 \pm 0.20

* "Freshly prepared" is used to designate solutions which had the PdCl₂ added immediately prior to the placement in measuring vessels.

TABLE 3
ABSOLUTE H₂ ADSORPTION BY "STANDING"^a AND "FRESHLY PREPARED" PVA-Pd AND GA-Pd CATALYSTS

CATALYST SOLUTION	CC. H ₂ ADSORBED	
	0.5%	1%
GA-Pd (standing)	4.10 \pm 0.20	4.10 \pm 0.20
GA-Pd (fresh)	4.10 \pm 0.20	4.10 \pm 0.20
PVA-Pd (standing)	2.30 \pm 0.20	0.00
PVA-Pd (fresh)	3.85 \pm 0.20	4.05 \pm 0.20

^a "Standing" is used to designate solutions wherein the PVA and PdCl₂ stood in contact at least 17 hours before transfer was made to the measuring vessels.

On analysis of these figures it is evident that the adsorptions observed in PVA-Pd solutions which are "freshly prepared," approximate those shown by GA-Pd. However, in "standing" PVA-Pd solutions a much smaller hydrogen adsorption than in GA-Pd was measured, and the 1% "standing" PVA-Pd showed no actual hydrogen adsorption at all, when allowance was made for the amount of hydrogen required for reduction.

The explanation of these anomalous results was that the PVA upon standing in contact with PdCl₂ caused a reduction of the PdCl₂ to metallic Pd. This would make intelligible the acquisition by the colloidal solutions on standing of a blackish shade. It appears from the data that 1% PVA is able to accomplish this stoichiometrically whereas 0.5% PVA does not fully reduce PdCl₂, only effecting a partial reduction in the overnight time interval.

This explained the apparent non-adsorption of hydrogen by "standing" PVA (1%)-Pd solutions, while a less active catalyst GA-Pd exhibited an adsorption of 4 cc.

Thus there was no significant difference in hydrogen adsorption displayed by PVA-Pd or GA-Pd solutions. Therefore, by this procedure no differences in particle size or available surface could be established between GA-Pd and PVA-Pd to explain the superiority of the PVA-Pd catalyst.

Additional proof for the fact that PVA reduced the PdCl₂ to Pd is afforded by the observation that upon determining the pH of the PVA-PdCl₂ solution immediately after mixing and then after standing, an increase in acidity was noted, due to the formation of HCl by the reduction of PdCl₂.

Ultracentrifugation and Rate of Hydrogenation.—Meanwhile, the problem of the relationship between particle size and rate of catalytic hydrogenation had been attacked from a different angle. The method of approach in this instance was to ultracentrifuge colloidal PVA-Pd solutions at various speeds, thus effecting a separation of the colloid into small Pd particles which would remain in the supernatant liquid and into the large particles which would be precipitated by the centrifuging. By quantitative deter-

mination of the Pd present in supernatant liquid and in residue and then by measuring the hydrogenation rates of equivalent quantities of Pd from both, an insight could be obtained into the effect of particle size and available surface on the activity of the catalyst. Through the courtesy of Dr. R. W. G. Wyckoff, of Lederle Laboratories at Pearl River, New York, PVA (1%)-Pd solutions were ultracentrifuged at 200 and 500 r. p. s. There was a noticeable difference in the shade of the two supernatant liquids, that which was centrifuged at the higher speed being much lighter.

The supernatant liquids were analyzed in the following way: A 1% PVA solution, which had been centrifuged at 500 r. p. s., was analyzed to determine the quantity of PVA in the top one-third and in the bottom one-third. Two cubic centimeters of the top one-third contained 18.02 mg. of total solid. The bottom one-third contained 20.50 mg. of solid per 2 cc. of solution. This gave a total solid in the two portions of 38.52 mg. per 4 cc. instead of the theoretical 40.00 mg. for a 1% solution. The total solids were then ignited for an hour to give 1.76% ash. This percentage of ash was then used to determine the quantity of Pd present in 5 cc. of supernatant liquid from the PVA-Pd solutions centrifuged at 200 and 500 r. p. s. Five cubic centimeters of supernatant liquid were taken to dryness and weighed to give total solids. Total solids were ignited and weighed to give "ash." This "ash" was in fact ash plus Pd. The weight of ash present could be obtained by multiplying the total solids by the percentage of ash previously found, i.e., 1.76%. The weight of Pd could then be determined by subtracting the weight of the ash thus obtained from the weight of Pd plus ash.

TABLE 4

DETERMINATION OF MG. OF Pd PRESENT IN 5 CC. OF SUPERNATANT LIQUID AT DIFFERENT RATES OF ULTRACENTRIFUGATION

SAMPLE	TOTAL SOLID	Pd + ASH IN MG.	% ASH IN PVA	ASH IN MG. (TOTAL SOLID X % Ash)	Pd IN MG. (ASH + Pd)—ASH
5 cc. (S. S.) ^a at 200 r. p. s.	44.43	1.73	1.76	0.78	0.95
5 cc. (S. S.) at 500 r. p. s.	43.94	1.28	1.76	0.77	0.51

^a S. S. stands for supernatant solution.

From the data it is seen that there was twice as much Pd present in the supernatant liquid of the sample ultracentrifuged at 200 r. p. s. as there was in the sample centrifuged at 500 r. p. s. Using these data, it was possible to set up a kinetic experiment in which the hydrogenation rate of equivalent quantities of Pd of different particle size could be compared; it is self-evident, of course, that the size of the Pd particles in the sample

centrifuged at 500 r. p. s. was smaller than that of the particles in the sample centrifuged at 200 r. p. s. If volumes of the supernatant liquids of the two centrifuged samples were taken which contained equivalent quantities of Pd, the hydrogenation rate of the supernatant liquid from the sample centrifuged at 500 r. p. s. should be greater than that of the sample centrifuged at 200 r. p. s. because of the greater surface of the former. This assumption, which correlates catalyst efficiency and particle size, proved correct.

The hydrogenation rates obtained with these two catalyst solutions containing equivalent quantities of Pd, i.e., 2 mg., are shown in figure 1, where it is demonstrated that the larger surface area in the 500 r. p. s. centrifuged sample is responsible for a greater efficiency of the catalyst.

Next the residues from the ultracentrifuging were analyzed to determine their Pd content so that aliquots containing 2 mg. of Pd could be taken and then compared with the activity of the particles of much smaller size in the supernatant liquids whose hydrogenation rates were just established and plotted in figure 1.

The results of analyses are recorded in table 5.

TABLE 5					
32-CC. SAMPLE CENTRIFUGED	Pd IN 32 CC.	VOL. OF S. S. ^a	MG. Pd PER 5 CC. S. S.	TOTAL MG. Pd IN S. S.	MG. Pd IN RESIDUE
200 r. p. s.	12.48	27.0	1	5.40	7.08
500 r. p. s.	12.48	26.4	0.5	2.64	9.84

^a "S. S." is used to designate the supernatant solution.

In order to conveniently apply the sediments, 5 cc. of distilled water were added to each bringing the volumes up to 6.3 cc. and 5.1 cc. for the 500 r. p. s. and 200 r. p. s., respectively. Thereby, 9.84 mg. of Pd and 7.08 mg. of Pd were present in 6.3 cc. and 5.1 cc., respectively. By calculation, the proportional volumes containing 2 mg. of Pd were determined and it was found that 1.28 cc. of the 500 r. p. s. sediment solution and 1.44 cc. of the 200 r. p. s. sediment solution were required. These volumes were then made up to 40 cc. in the same manner as were the supernatant liquids.

From figure 1, a convincing view of the effect of particle size on the hydrogenation rate may be obtained. For therein is presented the hydrogenation velocity toward a common substrate of analogous colloidal solutions of PVA-Pd catalysts containing equivalent quantities of Pd, viz., 2 mg. The difference between the rates of supernatant liquid and sedimented residues is striking. In the 500 r. p. s. sample, the sedimented material is about 13 times less active than the supernatant liquid using the adsorption of hydrogen at the end of five minutes as a basis for comparison: using fifteen minutes' adsorption as a basis, the sedimented material is about $7\frac{1}{2}$ times less active. With the 200 r. p. s. sample, the sediment

is about 5 times less active than the supernatant using the adsorption at the end of five minutes as a basis of comparison; using fifteen minutes' adsorption as a basis, it is about $4\frac{1}{3}$ times less active. Certainly, these ratios may only be attributed to the differences in surface area of the particles.

It was understood that the concentrations of PVA in the residue and in the supernatant solution were different, viz., 25 mg. and 90 mg., respec-

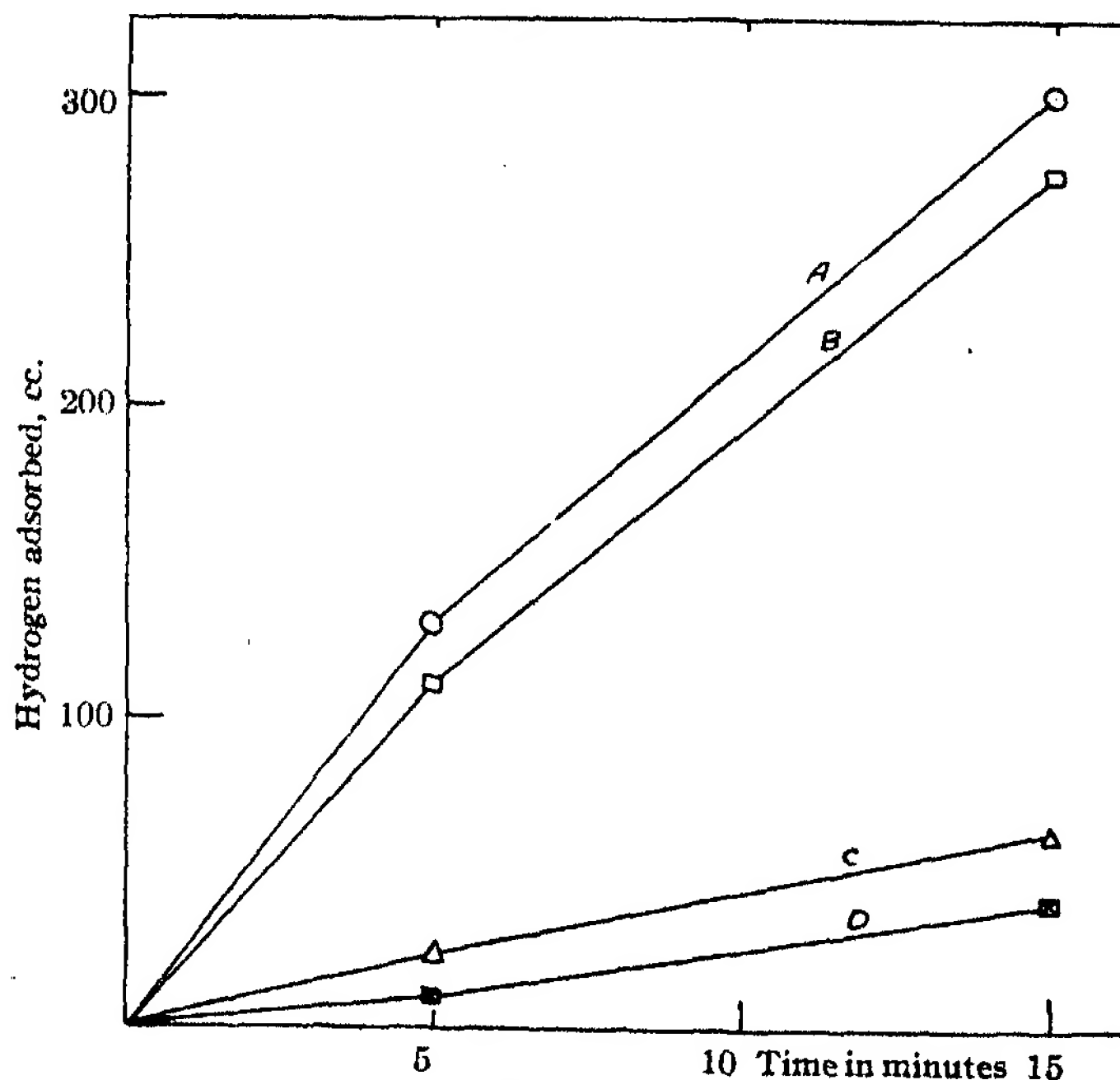


FIGURE 1

Comparison of Supernatant Liquids and Residues

Substrate: 2 mg. Pd in 40 cc. of 50% alcohol. Acceptor: 0.5 cc. $C_6H_5NO_2$. A, supernatant liquid of sample centrifuged at 500 r. p. s. B, supernatant liquid of sample centrifuged at 200 r. p. s. C, residue of catalyst centrifuged at 200 r. p. s. D, residue of catalyst centrifuged at 500 r. p. s. All measurements are converted to 25°C. and 760 mm.

tively, in the 200 r. p. s. centrifuged sample and 21 mg. and 175 mg., respectively, in the 500 r. p. s. centrifuged sample. From the data obtained by Rampino,⁶ it can be seen that the effect of PVA concentration would be insufficient to explain the striking differences in the measured efficiencies of the palladium in the residue- and supernatant-catalyst.

The superiority of the supernatant liquid of the 500 r. p. s. centrifuged sample to the supernatant liquid of the 200 r. p. s. sample is also noteworthy.



FIGURE 2

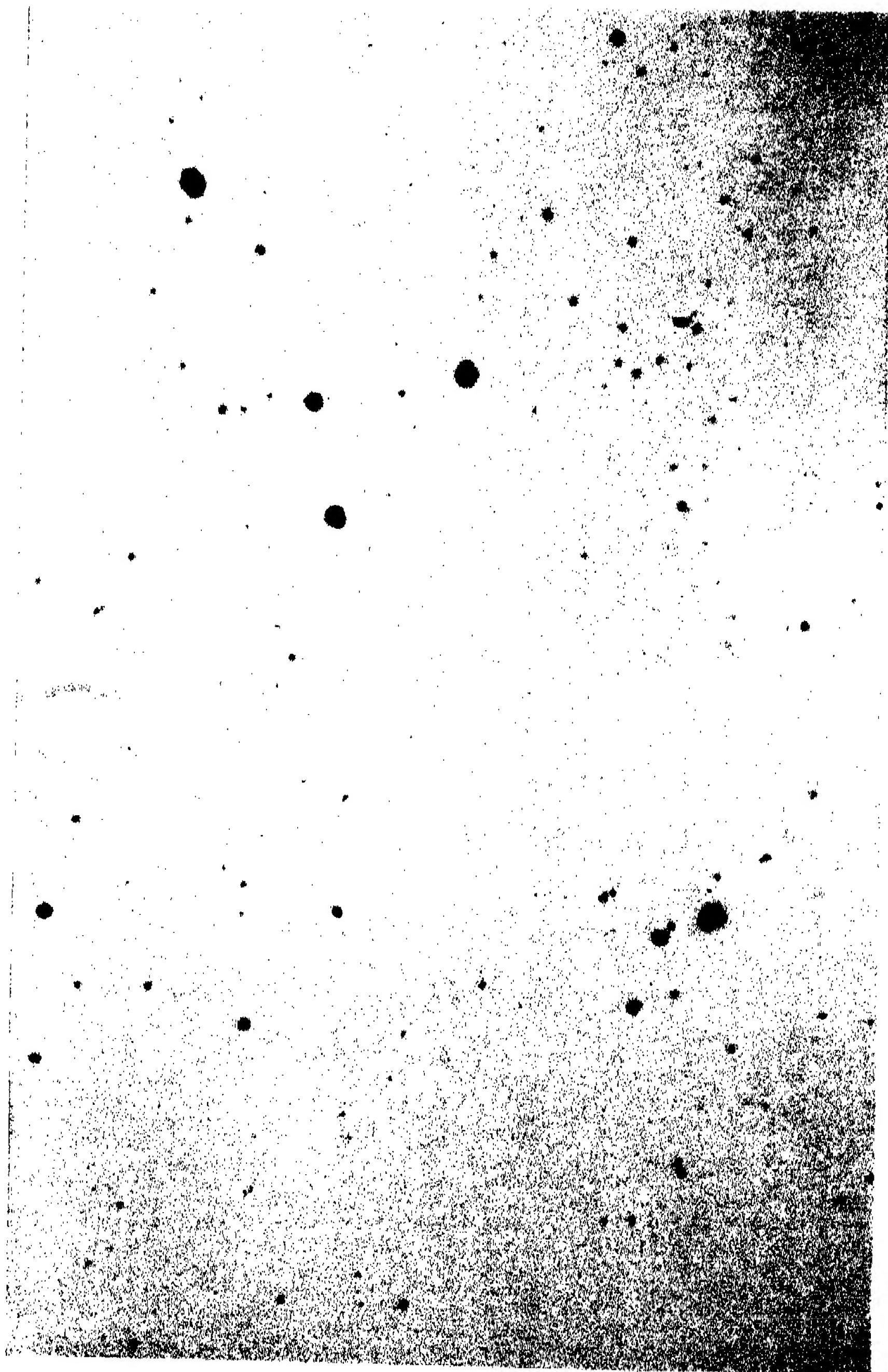


FIGURE 8

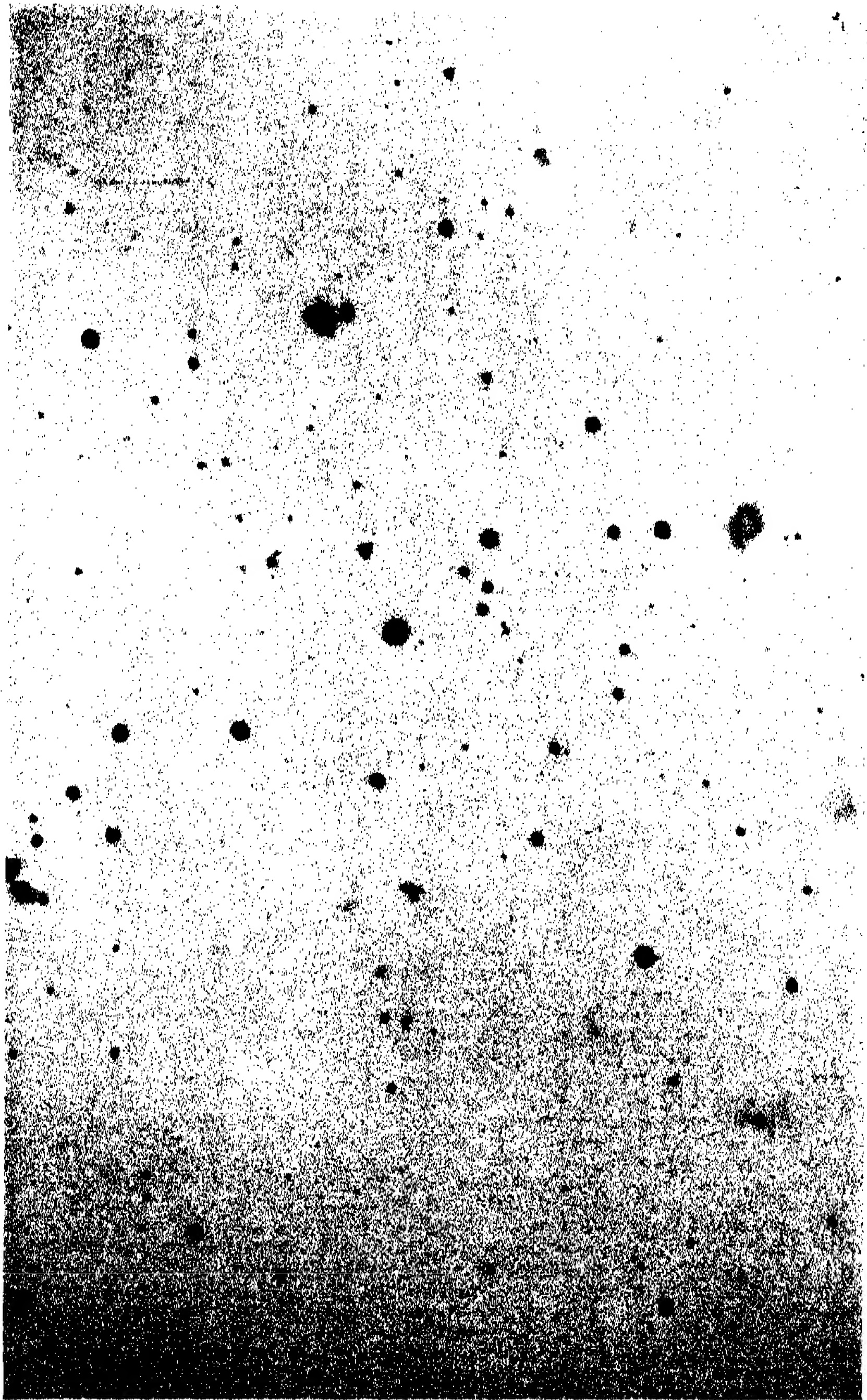


FIGURE 4

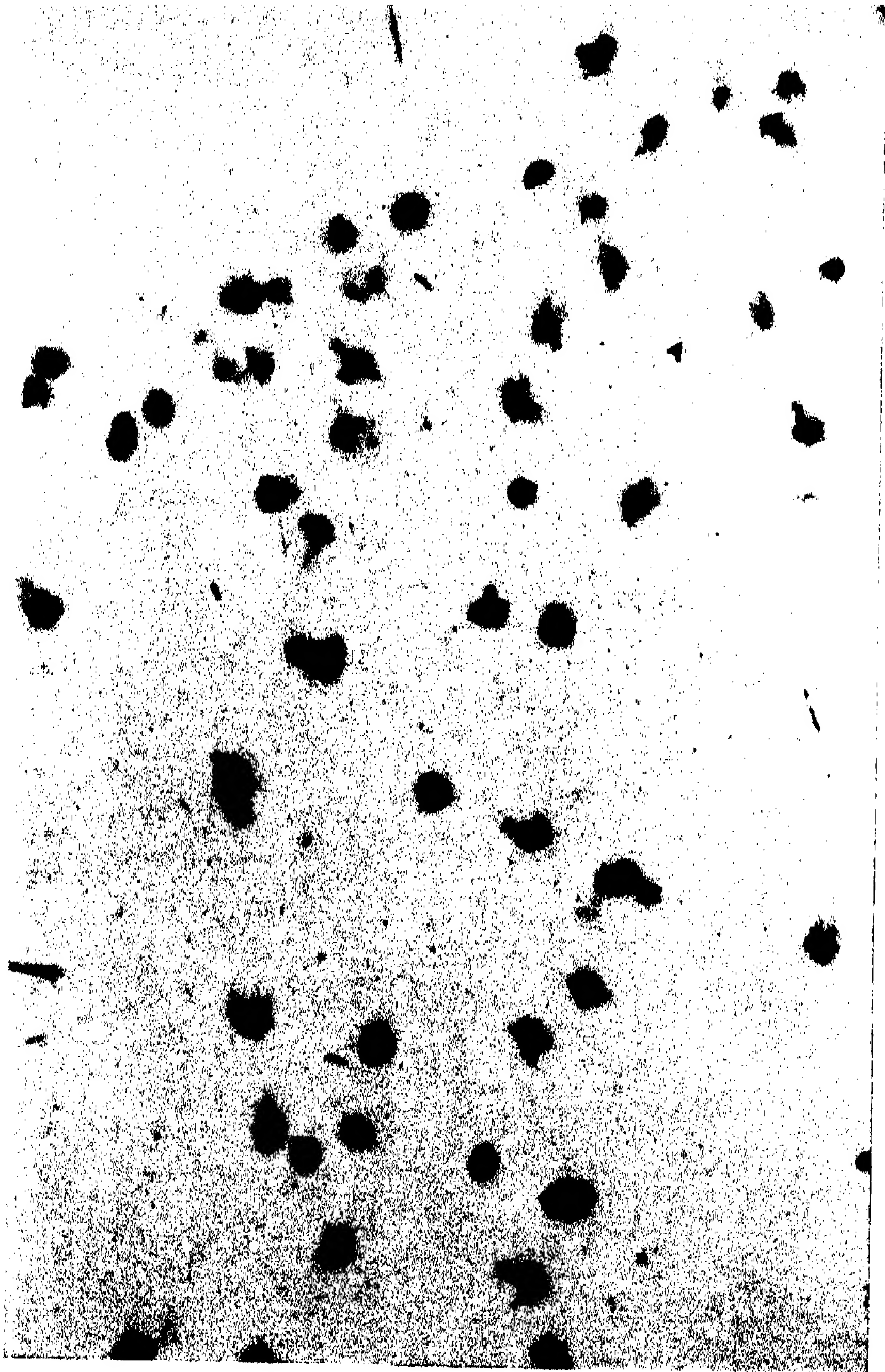


FIGURE 5

It appears from these results, that the bulk of catalytic activity rests preponderantly with the smaller particles. Thus surface area and particle size play a paramount rôle in hydrogenations performed with PVA-Pd catalysts.

Correlation of Electron Micrograph⁵ Measurements and Kinetic Experiments.—Since no significant differences in particle size could be established by the gas consumption experiments previously described, it was deemed necessary to resort to a more accurate and direct means of measuring the particle size, viz., by applying the electron microscope.

Figure 2 represents an electron micrograph of PVA alone. This showed a tendency to crystallize in acicular crystals of from 0.03 to 0.4 micron in length; the shape and density of these crystals was very different from that of the Pd seen in later micrographs. This micrograph was obtained from 0.5% PVA solution magnified 30,000 times.

In figures 3 and 4 two PVA-Pd catalysts are shown. They are identical in composition: they are both 0.5% in PVA (Du Pont's-Rh-391) and contain 10 mg. of Pd per 50 cc. of H₂O. They only vary in the method used to reduce the palladium chloride to metallic Pd. The catalyst shown in figure 3 has been reduced by hydrogen, i.e., by introducing the aqueous PVA-PdCl₂ solution into a vessel and shaking for five minutes. The catalyst shown in figure 4 has been reduced by PVA itself, i.e., by letting the PdCl₂ stand in contact with the PVA whereby the slow reduction of the PdCl₂ to Pd, takes place. This micrograph, with its characteristic Pd particles clearly visible, conclusively proves the fact that PVA reduces PdCl₂ to Pd, which was proposed to explain the anomalous gas adsorption results.

In these two micrographs, the black dense metallic Pd particles are clearly visible in hexagonal crystals, in which allotropic form palladium is known to exist.⁶ By far the greater number of particles assume an ultra-microscopic hexagonal configuration but some triangular shaped crystals can be noted. In these micrographs, the magnification is also 30,000 diameters. In both of these, there is no evidence of the PVA with its characteristic acicular crystals.

The possibility had been considered that these two PVA-Pd catalysts might have different hydrogenation efficiencies. The different modes of reduction could have produced Pd particles of varying sizes and of different surface areas and consequently two catalysts of varying effectiveness could have been formed. This seemed probable since one method of reduction, viz., by means of hydrogen, was of short duration, about five minutes, while the other, viz., by means of PVA, took in the case of 0.5% PVA, more than twenty hours. Kinetic experiments were run in an attempt to establish whether this difference existed. However, no significant difference could be established. Both catalysts gave hydrogenation rates of the same magnitude, within the experimental error, using nitrobenzene as an acceptor.

A statistical evaluation, wherein a size frequency curve of the single particles is determined from the electron micrographs of these two catalysts provides an explanation of their identity in kinetic behavior. A size frequency curve on the micrograph in figure 3, i.e., the PVA-Pd catalyst in which the PdCl_2 was reduced to Pd by hydrogen, gave a \bar{d}_s size⁷ (average particle size with respect to surface) of 0.07 micron. The same type of

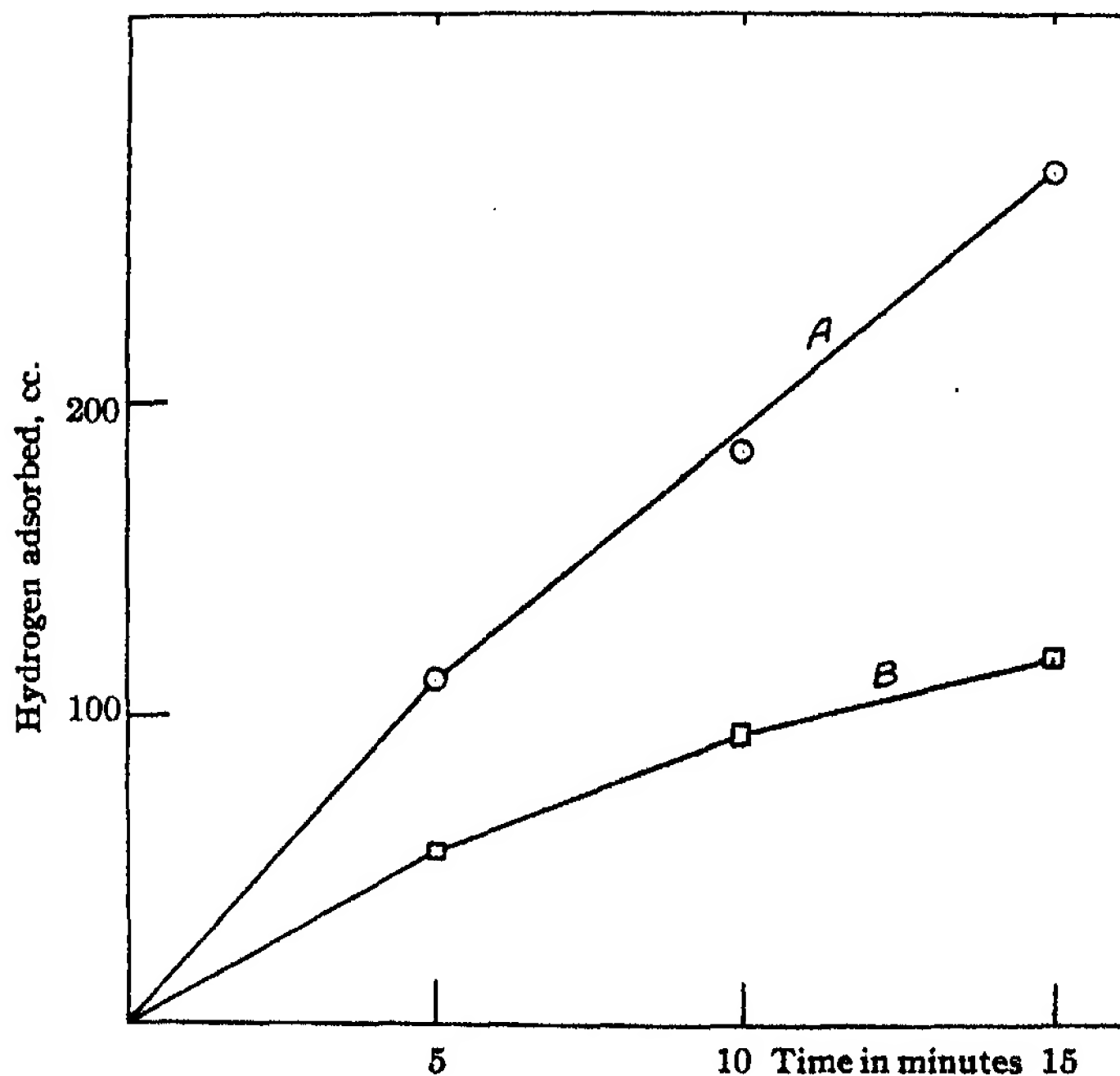


FIGURE 6

Comparison of "Supernatant" and "Normal" Catalysts

Substrate: 2 mg. Pd per 40 cc. of 50% alcohol. Acceptor: 0.5 cc. $\text{C}_6\text{H}_5\text{NO}_2$. A, supernatant liquid of PVA-Pd centrifuged at 200 r. p. s. B, normal H_2 -reduced PVA-Pd. All measurements are converted to 25°C. and 760 mm.

analysis on the figure 4 micrograph, i.e., the PVA-Pd catalyst in which the PdCl_2 was reduced to Pd by PVA itself, gave a \bar{d}_s size of 0.08 micron. There is only a slight difference in particle size of these two differently reduced catalysts, and this difference could not be expected to reveal significant deviations in the hydrogenation velocities produced by the two catalysts. Once again a relationship between surface area and catalyst efficiency is established.

Figure 5 is an electron micrograph, at a magnification of 30,000, of the

supernatant liquid of the PVA-Pd catalyst which was ultracentrifuged at 200 r. p. s. This micrograph was not made until a year and a half after the sample had been prepared and ultracentrifuged. As would be expected of a colloid which had been standing for such a length of time, a great deal of clustering had taken place, which is easily discernible in the micrograph.

However, the presence of very fine grain Pd is unmistakable. An estimate of the \bar{d}_s size amounts to 0.009 micron. This value might have to be revised slightly upward because the larger particles cannot be accurately measured in the clusters. There can be no doubt, moreover, that the particle size of much of the Pd is smaller than in the previous micrographs. Using the approximation of 0.009 as the \bar{d}_s size, the particle size seems to be about one-eighth that of the PVA-Pd H₂ reduced catalyst, wherein the \bar{d}_s size was shown to be 0.07 micron by a size frequency curve.

It has been previously described how a kinetic experiment was run on the supernatant solution of this 200 r. p. s. sample. For comparison purposes, the hydrogenation velocity of a non-centrifuged H₂-reduced PVA-Pd catalyst, containing 2 mg. of Pd and of similar composition to the catalyst prepared from the supernatant solution of the 200 r. p. s. sample, was measured.

These two hydrogenation velocities are plotted in figure 6.

It can be seen that the "supernatant" catalyst containing the same quantity of Pd as the analogous "normal" catalyst is more than twice as efficient. The analogous "normal" catalyst had a particle size about 8 times larger than that of the "supernatant" catalyst; 0.07 micron (statistically determined) to 0.009 micron (estimated). From the results of these experiments, it is evident that there is a definite relationship between the surface area of the catalyst and rate of hydrogenation.

The only two organic colloidal catalysts to be investigated by von Ardenne⁸ were Pt-Paal (sodium lysalbinat) and Pt-Skita (GA). He estimates from his micrographs that the particle size in the former is about 0.003 to 0.01 micron and in the latter, it averages about 0.025 micron. Of course, these measurements are made on platinum catalysts but it is reasonable to assume that the Pd particle size in these colloids would be of a comparable magnitude. If such an assumption is valid, certain conclusions may be drawn. The particle size of Pt and Pd, too (by analogy), in these two colloidal catalysts may be smaller, according to von Ardenne's measurements, than the particle size in PVA-Pd as determined from our micrographs; the Pt-Skita (GA) having a particle size diameter of 0.025 micron (estimated) to the 0.069 micron (calculated) diameter of PVA-Pd particles. In spite of this, the superiority of the PVA-Pd catalyst has been established. Also to be recalled is that no difference in particle size could be revealed by the gas adsorption measurements previously described. Consequently, although the preponderant effect of the particle size on the rate of hydro-

genation is definitely established, there seems to be another factor, a quality factor, the extent of which is, so far, not estimable, which is independent of particle size and which makes the synthetic high polymer carrier far superior to other colloidal carriers, e.g., GA, even though the particle size in the two types of carriers may be the same.

Whether the striking formation of ultramicroscopic hexagonal and triangular shaped Pd metal crystals in the presence of PVA has bearing on the original cause of the effectiveness of this type of catalyst, could be, perhaps, decided by comparing electron micrographs of catalysts deposited on solid bodies.

Summary.—By coördination of measurements of hydrogen adsorption, rates of hydrogenation and determination of the size and shape of catalyst particles it was established that the preponderance of catalytic activity lays with the Pd particles of smaller size. However, the superiority of the synthetic polymer supported catalyst over the GA type is to be attributed to an additional quality factor.

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† Present address: Shell Development Co., Emeryville, Calif.

‡ Communication No. 30.

§ Our appreciation is expressed to Mr. Ernest F. Fullam of Interchemical Corporation of New York for his courtesy in preparing numerous electron micrographs and a discussion of the results, and to Baker and Co., Inc., Newark, N. J., for donating the noble metal salts used in this investigation.

¹ Leichter, H., and Nord, F. F., *Biochem. Z.*, **295**, 226 (1938).

² Rampino, Louis D., and Nord, F. F., *Jour. Amer. Chem. Soc.*, **63**, 2745, 3268 (1941).

³ Kavanagh, Kevin E., *Ibid.*, **64**, 2721 (1942); Rampino, Louis D., and Nord, F. F., *Ibid.*, **65**, 429 (1943).

⁴ Beeck, O., Smith, A. E., and Wheeler, A., *Proc. Roy. Soc. (London)*, **A117**, 62 (1940).

⁵ Nord, F. F., *Ber.*, **52**, 1705 (1919).

⁶ Dissertation, Fordham University, 1941, figure 5.

⁷ Naumann, C. F., and Zirkel, F., *Elemente der Mineralogie*, 15th edition, Leipzig, Engelmann, 1907, p. 421.

⁸ Green, H., *Jour. Franklin Inst.*, **204**, 713 (1927).

⁹ von Ardenne, M., and Beischer, D., *Angew. Chem.*, **53**, 103 (1940).

*THE DETERMINATION OF L.D.50 AND ITS SAMPLING ERROR
IN BIO-ASSAY, III*

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Communicated June 24, 1943

1. The discussion of this problem has been conducted on the hypothesis that the number n of animals used in each of the dilutions is the same. This restriction is convenient in that it leads to the possibility of preparing a table for the determination of the constants and of their standard errors when there are three dilutions.¹ Formulae can, however, be developed for the more general case where the numbers are not the same, but n_1, n_2, n_3 . The rule of maximum likelihood leads to the equations:

$$\frac{\partial L}{\partial \gamma} = -\alpha \Sigma(2s_i - n_i) + \alpha \Sigma n_i \tanh \alpha(x_i - \gamma) = 0, \quad (1)$$

$$\frac{\partial L}{\partial \alpha} = \Sigma(2s_i - n_i)(x_i - \gamma) - \Sigma n_i(x_i - \gamma) \tanh \alpha(x_i - \gamma) = 0. \quad (2)$$

Placing x_1, x_2, x_3 equal to $x - c, x, x + c$ and setting the derivatives equal to zero, introducing $X = \tanh \alpha(x - \gamma)$ and $C = \tanh \alpha c$ we have the simultaneous equations for X and C

$$C^2[n_2X^3 - \mathbf{A}X^2 + (n_1 + n_3)X] + C(n_3 - n_1)(X^2 - 1) + \mathbf{A} - NX = 0, \quad (3)$$

$$C^2[(n_3 - n_1)X - 2\mathbf{B}X^2] + C(n_3 + n_1)(X^2 - 1) + 2\mathbf{B} - (n_3 - n_1)X = 0, \quad (4)$$

where

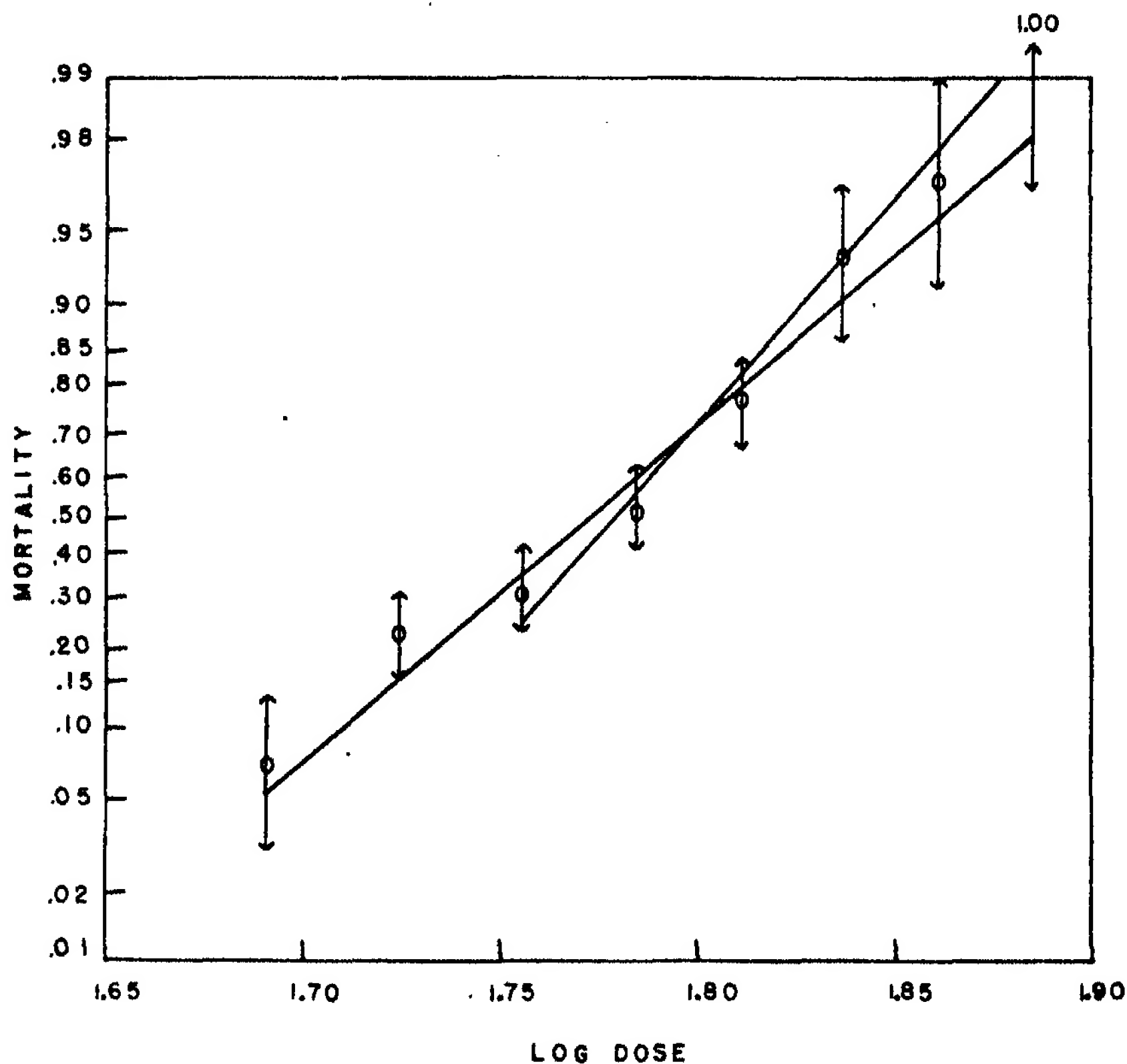
$$\mathbf{A} = 2(s_1 + s_2 + s_3) - N, \quad 2\mathbf{B} = 2s_3 - 2s_1 - n_3 + n_1 \quad (5)$$

and $N = n_1 + n_2 + n_3$ is the total number of animals used. Here \mathbf{A} and \mathbf{B} which take the place of A and B in the earlier treatment are in terms of numbers, not of proportions, of the animals affected. The elimination of C gives a cubic equation for X only slightly more complicated than that found before, viz.,

$$\begin{aligned} Nn_2X^3 - [\mathbf{A}(N + n_2) - 2\mathbf{B}(n_3 - n_1)]X^2 \\ + [\mathbf{A}^2 - 4\mathbf{B}^2 + N(n_3 + n_1) - (n_3 - n_1)^2]X \\ - [\mathbf{A}(n_3 + n_1) - 2\mathbf{B}(n_3 - n_1)] = 0. \end{aligned}$$

Having solved this numerical equation for X , one may obtain C from the equation

$$C = \frac{-A(n_3 - n_1) + 2B(n_3 + n_1) + n_2(n_3 - n_1) X}{n_2(n_3 + n_1)X^2 - [A(n_3 + n_1) - 2B(n_3 - n_1)]X + 4n_1n_3},$$



and then

$$\alpha = \frac{1}{c} \tanh^{-1} C, \quad \gamma = x - \frac{1}{\alpha} \tanh^{-1} X,$$

where the functions \tanh^{-1} may be replaced by their equivalents in logarithms.

The sampling errors may be obtained from

$$\frac{\partial^2 L}{\partial \gamma^2} = -\alpha^2 \sum n_i \operatorname{sech}^2 \alpha(x_i - \gamma) = -4\alpha^2 \sum n_i P_i Q_i,$$

$$\frac{\partial^2 L}{\partial \alpha^2} = -\sum n_i (x_i - \gamma)^2 \operatorname{sech}^2 \alpha(x_i - \gamma) = -4\sum n_i P_i Q_i (x_i - \gamma)^2,$$

$$\frac{\partial^2 L}{\partial \alpha \partial \gamma} = \alpha \sum n_i (x_i - \gamma) \operatorname{sech}^2 \alpha(x_i - \gamma) = 4\alpha \sum n_i P_i Q_i (x_i - \gamma),$$

when P_i are the fitted values, by

$$\sigma_\alpha^2 = -\frac{1}{H} \frac{\partial^2 L}{\partial \gamma^2}, \quad \sigma_\gamma^2 = -\frac{1}{H} \frac{\partial^2 L}{\partial \alpha^2},$$

where H is the Hessian of L with respect to γ and α . As a matter of fact these formulae are valid for any number of dilutions provided a solution for α and γ and consequently for P_i upon the curve has been found in any manner, presumably by successive approximation from the equations

$$\left. \frac{\partial L}{\partial \gamma} \right|_0 + \left. \frac{\partial^2 L}{\partial \gamma^2} \right|_0 \delta \gamma + \left. \frac{\partial^2 L}{\partial \alpha \partial \gamma} \right|_0 \delta \alpha = 0, \quad \left. \frac{\partial L}{\partial \alpha} \right|_0 + \left. \frac{\partial^2 L}{\partial \alpha \partial \gamma} \right|_0 \delta \gamma + \left. \frac{\partial^2 L}{\partial \alpha^2} \right|_0 \delta \alpha = 0.$$

2. In case one is working with a sufficient number of dilutions and in case the observed values P_i do not fluctuate too much from a straight line on growth paper one may get an approximate solution by graphical methods. For example, if we have the following logarithmic doses x and proportions of population affected²

x	1.755	1.784	1.811	1.837	1.861	1.884
n	28	27	30	31	30	29
s	9	14	23	29	29	29
P	0.329	0.519	0.767	0.936	0.967	1.000

we may plot the first 5 points ($P = 0$ or 1.0 cannot be plotted) and draw the line. The 50% end-point is read as at 1.777. The fitted growth curve being

$$P_f = \frac{1}{2} + \frac{1}{2} \tanh \alpha (x - 1.777), \quad \alpha = \frac{\tanh^{-1}(2P-1)}{x - 1.777}$$

permits the determination of α from any values P_f and x read from the line. At $x = 1.837$, $P_f = 0.935$. Hence $\alpha = 22.2$ and $P_f = \frac{1}{2} + \frac{1}{2} \tanh 22.2 (x - 1.777)$ is the line as determined graphically.

3. The values of P_f as fitted may be read³ from the line and the calculation form may be filled out.

x	1.755	1.784	1.811	1.837	1.861	1.884
P_f	0.280	0.587	0.810	0.935	0.975	0.992
Q_f	0.720	0.413	0.190	0.065	0.025	0.008
$P_f - P$	-0.049	0.068	0.043	-0.001	0.008	-0.008
$s_f - s$	-1.37	1.84	1.29	-0.03	0.24	-0.23
$x - \gamma$	-0.022	0.007	0.034	0.060	0.084	0.107
$(s_f - s)(x - \gamma)$	0.0301	0.0129	0.0439	-0.0018	0.0202	-0.0246
$4nP_fQ_f$	22.6	26.1	18.5	7.5	2.9	0.9
$4nP_fQ_f(x - \gamma)$	-0.50	0.18	0.63	0.45	0.25	0.10
$4nP_fQ_f(x - \gamma)^2$	0.011	0.001	0.021	0.027	0.021	0.011

Now,

$$\left. \frac{\partial L}{\partial \gamma} \right|_0 = 2\alpha \Sigma(s_f - s) = 77.3, \quad \left. \frac{\partial L}{\partial \alpha} \right|_0 = -2\Sigma(s_f - s)(x - \gamma) = -0.161,$$

$$\left. \frac{\partial^2 L}{\partial \gamma^2} \right|_0 = -38,700, \quad \left. \frac{\partial^2 L}{\partial \alpha^2} \right|_0 = -0.092, \quad \left. \frac{\partial^2 L}{\partial \alpha \partial \gamma} \right|_0 = 24.6.$$

Then $77.3 - 38,700\delta\gamma + 24.6\delta\alpha = 0$, $-0.161 + 24.6\delta\gamma - 0.092\delta\alpha = 0$ give as solutions $\delta\gamma = 0.00106$, $\delta\alpha = -1.47$ and $\gamma = 1.77806$, $\alpha = 20.73$ as a first arithmetic approximation subsequent to the graphical fit. If the graphical fit be considered an adequate approximation for computing σ_γ and σ_α we have for the Hessian $H = 2955$ and hence

$$\sigma_\alpha^2 = \frac{38,700}{2955} = 13.1, \quad \sigma_\gamma^2 = \frac{0.092}{2955} = 0.000031,$$

which gives $\sigma_\alpha = 3.6$, $\sigma_\gamma = 0.0056$ and we write⁴

$$\gamma = 1.7781 \pm 0.0056, \quad \alpha = 20.7 \pm 3.6.$$

In this example the logarithmic doses x did not advance by uniform amounts so that it is impossible satisfactorily to determine α and γ from the table which was computed on the basis of an equal advance in x and constant values of n ; but if one should ignore these points and take the 1st, 3rd and 5th observed P 's we should find $A = 1.126$, $B = 0.638$ giving " γ " = -0.63 and " α " = 1.00. With $c = 0.053$ we have $\gamma = -0.033$ and $\alpha = 19$. The 50% end-point would be at $1.811 - 0.033 = 1.778$. The values 1.778 and 19 in place of 1.777 and 22.2 determined graphically or 1.7781 and 20.7 obtained by the first arithmetic approximation are much the same thing in view of the standard errors estimated from the graphical solution. We could not expect to get the values of the standard deviations from the table because they would be estimated from only about one-half of the observations and furthermore the estimates depend on the actual points used on the growth curve as well as on the number of observations; in fact a calculation gives 1.778 ± 0.0080 and 19 ± 4.2 in place of 1.7781

± 0.0056 and 20.7 ± 3.6 . In this case, however, the use of the table and only three of the six observed proportions gives a fairly good answer.

4. There need be no restriction to three points or to identical values of n_i . If in (1) and (2) we write $x_i = x + ic$, assuming the logarithmic doses to proceed in a uniform manner (or the dilutions to be a geometric progression) we have for the determination of γ and α

$$\begin{aligned} -\alpha \Sigma(2s_i - n_i) + \alpha \Sigma n_i \tanh \alpha(x + ic - \gamma) &= 0, \\ \Sigma(2s_i - n_i)(x + ic - \gamma) - \Sigma n_i(x + ic - \gamma) \tanh \alpha(x + ic - \gamma) &= 0. \end{aligned}$$

If the first equation be multiplied by $(x - \gamma)/\alpha$ and added to the second, the latter becomes

$$\Sigma i(2s_i - n_i) - \Sigma i n_i \tanh \alpha(x + ic - \gamma) = 0.$$

The equations will be algebraic in $X = \tanh \alpha(x - \gamma)$ and $C = \tanh c$ but of too high an order to be readily used. It will, however, be noticed that the solution depends on the particular two constants

$$A = \Sigma(2s_i - n_i), \quad 2B = \Sigma i(2s_i - n_i).$$

For this reason, if some standard set-up involving more than three dilutions in geometric progression were to be adopted for wide-spread use it would be possible, and might be well, to compute a double entry table for³ γ and α and their standard deviations in terms of **A** and **B**.

¹ These PROCEEDINGS, 29, 79-85, 114-120, 150-154, 207-212 (1943).

² For growth paper, see Wilson, E. B., these PROCEEDINGS, 11, 451-456 (1925). The data used in the illustration are taken from Bliss, C. I., p. 154 of *Ann. Applied Biol.*, 22, 134-167 (1935) or from Irwin, J. O., p. 17 of *Suppl. Jour. Roy. Stat. Soc.*, 4, 1-60 (1937). Those authors fit only the last six of the eight points. In the figure the limits of P at one standard deviation as determined from the formula on p. 209 of *Jour. Amer. Statis. Assoc.*, 22, 209-212 (1927) have been inserted and two lines have been drawn, one fitting (graphically) the last six and the other all eight points. It is clear that the graphical fit to the eight points is not bad; indeed if one actually fits the eight points arithmetically one finds $\gamma = 1.7714 \pm 0.0056$, $\alpha = 17.1 \pm 2.1$ with a χ^2 value of 3.35 which for six degrees of freedom gives $P = 0.76$ with a probability that because of the small numbers in some of the cells, the true value of P would be even larger. It is true that the deviations seem to be of such signs as to indicate curvature of the observed points to the fitted line, but in view of the small value of χ^2 and high value of P one might hesitate to draw that inference and to reject the first two points from this statistical evidence. The real reason for the rejection of these two points lies in the fact that in other corresponding sets of data the mortalities at low dosages seem also to be too high to fit the growth curve or probability integral curve. However, if the true form of the curve fitting such data were known, the values of the 50% end-point and of its standard error might be somewhat different from those found by fitting either of these curves to the last six points.

³ It might be better to calculate the values of P , from the analytic expression even for the first arithmetic approximation to improve the value graphically determined, and calculation would certainly be necessary for a second arithmetic approximation.

⁴ If the arithmetic approximations were continued we should find $\gamma = 1.7787$, $\alpha = 20.53$. The value of χ^2 would be such as to give $P = 0.92$ for the fit to the six points, which would indicate that the variance of the observed from the fitted points was only 0.29 of the amount which on the average would be due to chance or that the variation of the observed from the fitted points would be only 0.54 of the chance amount.

⁵ For the actual computation of such a table one would not go back to the general and approximative equations which one would use to determine α and γ for given values of **A** and **B**. As the values of **A** and **B** are the same for the observed data and for the values fitted to them, one would start with assumed values of " γ " and " α " and determine **A** and **B** from the equations.

$$A = \sum n_i \tanh \alpha (i - \gamma), \quad 2B = \sum i n_i \tanh \alpha (i - \gamma).$$

This would give a table for **A** and **B** in terms of " γ " and " α " from which the table for " γ " and " α " could be obtained by inversion.

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DYNAMICAL TRAJECTORIES IN A RESISTING MEDIUM

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1. In the Princeton Colloquium Lectures,¹ we discussed the motion of a particle in the plane under the action of any positional field of force. The general equations of motion are²

$$\ddot{x} = \phi(x, y), \quad \ddot{y} = \psi(x, y), \quad (1)$$

where (ϕ, ψ) are the rectangular components of the force acting at any point (x, y) .

The total number of trajectories, for all initial conditions, is ∞^3 . The differential equation of third order representing this system of trajectories (found by eliminating the time t from (1)) is

$$(\psi - y'\phi)y''' = [\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2]y'' - 3\phi y''^2. \quad (2)$$

This is not an arbitrary differential equation of the third order. We have given a completely characteristic set of *five* geometric properties of the dynamical trajectories in the Princeton Colloquium. The dynamical trajectories are projectively invariant, and recently Terracini has transformed each of the five properties into purely projective language.³

2. In the subsequent discussion, we shall need only three of the characteristic sets of five geometric properties. These may be stated in the following manner.

Property I. If for each of the ∞^1 trajectories passing through a given point in a given direction we construct the osculating parabola at the given point, the locus of the foci of these parabolas is a circle passing through that point.

The most general systems of ∞^3 curves possessing the Property I are given by differential equations of the forms⁴

$$(G): y''' = G(x, y, y')y'' + H(x, y, y')y'^2. \quad (3)$$

Property II. The circle that corresponds according to the Property I, to a lineal element, is so situated that the element bisects the angle between the tangent to the circle and a certain direction fixed for the given point (the direction of the force acting at the given point).

The most general systems with the properties I and II are defined by differential equations of the forms

$$(G_{II}): (y' - \omega)y''' = (y' - \omega)Gy'' + 3y''^2, \quad (4)$$

where G is any function of (x, y, y') , and ω is any function of (x, y) . The type (G_{II}) thus involves *one* arbitrary function of *three* arguments and *one* arbitrary function of *two* arguments.

Property III. In each direction at a given point there is one trajectory which has four-point contact with its circle of curvature: the locus of the centers of the ∞^1 hyperosculating circles constructed at the given point is a conic passing through that point in the fixed direction described in Property II.

Thus the most general systems of ∞^3 curves possessing the properties I, II and III, are defined by differential equations of the forms

$$(G_{III}): (y' - \omega)y''' = (\lambda y'^2 + \mu y' + \nu)y'' + 3y''^2, \quad (5)$$

involving *four* arbitrary functions $(\omega, \lambda, \mu, \nu)$ each of two arguments (x, y) .

If we impose the two additional geometric properties IV and V, we shall find that the type (G_{III}) will finally define the dynamical trajectories of a positional field of force. However, we shall not write the two properties IV and V as they are not necessary for our purpose in this paper.

3. We consider the motion of a particle moving in the plane under a positional field of force and influenced by a resisting medium, the resistance acting in the direction of the motion and varying as some function of the speed v . In ballistics, the resistance R is usually taken as an empirical function consisting of various powers of the speed v for different intervals of time. In our work, we shall determine the form of the resistance R in order that the trajectories in the resisting medium shall possess some of the properties possessed by those in the case of no resistance.

Let the resistance R be defined by the equation

$$R = vf(v). \quad (6)$$

The equations of motion will then be of the form

$$\ddot{x} = \phi(x, y) + \dot{x}f(v), \quad \ddot{y} = \psi(x, y) + \dot{y}f(v). \quad (7)$$

Upon eliminating the time t from these equations, we find that the differential equation of the trajectories is

$$(\psi - y'\phi)y''' = [\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2]y'' - 3\phi y''^2 - 2fy''^{1/2}(\psi - y'\phi)^{1/2}, \quad (8)$$

where the argument v of f is to be expressed in terms of (x, y, y', y'') by means of

$$v^2 = \frac{1}{y''} (1 + y'^2)(\psi - y'\phi). \quad (9)$$

4. We wish now to obtain a fundamental intrinsic representation of the system of ∞^3 trajectories in a positional field of force influenced by a resisting medium, the resistance R acting in the direction of the motion and varying as some function of the speed v . Upon decomposing the acting force into components N , normal, and T , tangential to the path, we find that the equations of motion (7) may be written in the form

$$v^2 = rN, \quad vv_s = T + R, \quad (10)$$

where v denotes the speed, s the arc length and r the radius of curvature. By differentiating the first of these equations with respect to s , and comparing with the second equation, we can eliminate v , obtaining

$$\frac{d}{ds} (rN) = 2T + 2R, \quad (11)$$

a relation which defines the trajectories and is equivalent to (8).

To reduce this to a more explicit form, we introduce an auxiliary vector, completely determined by the given field of force, namely the space derivative of the force (considered of course as a vector). The normal and tangential components of the original force vector are

$$N = \frac{\psi - y'\phi}{(1 + y'^2)^{1/2}}, \quad T = \frac{\phi + y'\psi}{(1 + y'^2)^{1/2}}; \quad (12)$$

the corresponding components of the new vector are

$$\begin{aligned} \mathfrak{N} &= \frac{\psi_s - y'\phi_s}{(1 + y'^2)^{1/2}} = \frac{\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2}{(1 + y'^2)}, \\ \mathfrak{T} &= \frac{\phi_s + y'\psi_s}{(1 + y'^2)^{1/2}} = \frac{\phi_x + (\phi_y + \psi_x)y' + \psi_y y'^2}{(1 + y'^2)}. \end{aligned} \quad (13)$$

While the new vector is the s derivative of the force vector, its components are obviously not the same as the s derivatives of the old components: the correct relations are found to be

$$N_s = \mathfrak{N} - T/r, \quad T_s = \mathfrak{T} + N/r. \quad (14)$$

These formulae are sufficient for the discussion of the trajectories. By means of (14) we can reduce (11) to the form

$$Nr_s = -r\mathfrak{N} + 3T + 2R. \quad (15)$$

This is the fundamental intrinsic representation of the system of ∞^3 trajectories connected with a given field of force influenced by a resisting medium, the resistance R acting in the direction of the motion and varying as some function of the speed v .

From the preceding result, we may obtain the following theorem.⁶ *If a particle starts from rest, the initial radius of curvature of the trajectory is to the radius of curvature of the line of force passing through the initial point as $(3T + 2R)$ is to T . In most instances, R vanishes (when $v = 0$) so that this ratio of the corresponding radii of curvature is 3:1.*

5. Consider now the ∞^1 trajectories starting from a given lineal element (x, y, y') . The focal locus, that is, the locus of the foci of the osculating parabolas, varies in shape with the function f , that is, with the law of resistance. This focal locus (X, Y) may be given parametrically by the complex equation

$$\frac{3(1 + iy')^2(\psi - y'\phi)}{2[(X + iY) - (x + iy)]} = (1 + iy')[\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2] - 3(\phi + i\psi)y'' - 2fy''^{1/2}(1 + iy')(\psi - y'\phi)^{1/2}. \quad (16)$$

We know that, if there is no resistance, Property I is valid, that is, the focal locus is a circle passing through the given point. Are there any resisting media for which this property is preserved? By comparing (8) with (3), it follows that the last term of (8) must be of the form $ay'' + by''^2$, where a and b are functions of (x, y, y') only. Hence it follows that f must be of the form $Av + B/v$, where A and B are constants. By (6), it follows that $R = Av^2 + B$.

The only appropriate media for which the Property I is valid are those for which the resistance R is of the form $Av^2 + B$, where A and B are constants.

This result could have been obtained also from the equation (15) by imposing the condition that r_s be linear integral in r . This will be the case if and only if $R = Av^2 + B$.

Now if the resistance R is $Av^2 + B$, the last term of (18) is of the form

$$- \frac{2}{(1 + y'^2)^{1/2}} [A(1 + y'^2)(\psi - y'\phi)y'' + By''^2]. \quad (17)$$

For such media, Property II will not usually be fulfilled. By comparing (8) together with (17) with equation (4), we see that the Property II will be fulfilled if and only if $B = 0$.

The only medium preserving the properties I and II is that in which the resistance varies as the square of the speed. This is the particular law studied by Newton in the Principia.

If we impose the Property III, both A and B must vanish, that is, the resistance vanishes and the force is purely positional.

6. It is of interest to examine the case where the resistance varies as any power v^n of the speed. By (8), the differential equation of the trajectories is

$$(\psi - y'\phi)y''' = [\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2]y'' - 3\phi y'^2 - 2k(1 + y'^2)^{(3-2m)/2}(\psi - y'\phi)^{2-m}y''^m, \quad (18)$$

where

$$m = \frac{1}{2}(4 - n). \quad (19)$$

By (16), the focal locus in this case is given parametrically by the single complex equation

$$\frac{3(1 + iy')^2(\psi - y'\phi)}{2[(X + iY) - (x + iy)]} = (1 + iy')[\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2] - 3(\phi + i\psi)y'' - 2k(1 + iy')(1 + y'^2)^{(3-2m)/2}(\psi - y'\phi)^{2-m}y''^{m-1}. \quad (20)$$

Therefore the focal locus is a curve whose inverse with respect to the given point is of the form

$$X = a + b(Y - y'X) + c(Y - y'X)^{m-1}, \quad (21)$$

where (a, b, c) are special functions of (x, y, y') only.

The focal locus is a straight line (as in the case of no resistance) when m is 1 or 2, that is, when n is 2 or 0.

The curve is a conic when m is 3, 0 or 3/2, that is, when n has one of the values -2 or 4 or 1. When $n = -2$ the conic is a parabola with its axis parallel to the given element. When $n = 4$ it is a hyperbola, asymptotic to the line of the given initial element. When $n = 1$ it is a parabola touching the initial line (not at the given point).

7. Corresponding results may be obtained in ordinary space. The only laws for which the spatial properties I and II (see the Princeton Colloquium) are valid are those included in $R = Av^2 + B$. If the spatial Property III is also preserved, the resistance must vanish. Property I is valid obviously for any medium.

¹ Kasner, "Differential-Geometric Aspects of Dynamics," *Amer. Math. Soc. Colloquium Publications*, 3 (1912, 1934). Also see a series of papers in *Trans. Amer. Math. Soc.*, 7-11 (1906-1910).

² Throughout this paper, dots indicate total differentiation with respect to the time t ; primes indicate total differentiation with respect to x ; subscripts x and y indicate partial differentiation; finally the subscript s indicates the total differentiation with respect to the arc length s .

³ Terracini, "Sobre la ecuacion diferencial $y''' = G(x, y, y')y'' + H(x, y, y')y'^2$," *Univ. Nac. Tucuman, Revista A*, 2, 245-329 (1941).

⁴ Any differential equation of the type (G) does define the ∞^3 dynamical trajectories of generalized fields of force depending not only upon the point but also upon the direction through the point. See Kasner and De Cicco, "A Generalized Theory of Dynamical Trajectories," *Trans. Amer. Math. Soc.*, July 1943.

⁵ This theorem has been generalized to acceleration fields of higher order. See Kasner and Mittleman, "A General Theorem on the Initial Curvature of Dynamical Trajectories," *Proc. Nat. Acad. Sci.*, 28, 48-52 (1942). Also De Cicco, "Extensions of Certain Dynamical Theorems of Halphen and Kasner," *Bull. Amer. Math. Soc.* (1943).

DYNAMICAL TRAJECTORIES OF THE CURVATURE TYPE¹

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1. Kasner has determined all those fields of force in the plane whose dynamical trajectories are of the curvature type. The appropriate fields are those of the central or parallel type.² In this paper, we propose to discuss this problem in three-dimensional space. We find that all fields of force in space whose dynamical trajectories are also curvature trajectories consist of three distinct types, one of which is the central or parallel type, and the other two are complicated and new.

2. Kasner in his Princeton Colloquium Lectures,³ studied the geometry of the dynamical trajectories in the plane and in space. In the plane, it is shown that the ∞^3 dynamical trajectories may be characterized by a set of *five* independent properties, whereas in space, the ∞^6 dynamical trajectories may be completely characterized by a set of *four* independent properties.

Consider the motion of a particle of unit mass in space under the action of any positional field of force. The general equations of motion are

$$\frac{d^2x}{dt^2} = \phi(x, y, z), \quad \frac{d^2y}{dt^2} = \psi(x, y, z), \quad \frac{d^2z}{dt^2} = \chi(x, y, z), \quad (1)$$

where (ϕ, ψ, χ) are the rectangular components of the force acting at any point (x, y, z) .

The total number of trajectories for all initial conditions, is ∞^6 . By eliminating the time t from these equations, it is found that the ∞^6 dynamical trajectories are represented by the differential equations

$$(\psi - y'\phi)z'' = (\chi - z'\phi)y'',$$

$$(\psi - y'\phi)y''' = [(\psi_x + y'\psi_y + z'\psi_z) - y'(\phi_x + y'\phi_y + z'\phi_z)]y'' - 3\phi y''^2. \quad (2)$$

3. The curvature trajectories of a given family of ∞^4 curves in space may be defined in the following way. A curvature trajectory of the given family is a curve which is drawn so that at each point it has the same osculating plane as, and also c times the curvature of the member of the family to which it is tangent at that point, c remaining constant along the trajectory. For a given value of c there will be a set of ∞^4 curvature trajectories, one in each direction through each point. By varying c , there result ∞^1 such sets. Hence a given quadruply-infinite family generates a quintuply-infinite family of curvature trajectories.

If the original family of ∞^4 curves does not consist entirely of straight lines, it may be shown that it is represented by the pair of Monge differential equations of second order

$$\log y'' = F(x, y, z, y', z'), \quad z'' = e^F K(x, y, z, y', z'), \quad (3)$$

where F and K are two functions in the five variables. The curvature trajectories of this family (3) of ∞^4 curves are then given by the pair of simultaneous differential equations

$$z'' = Ky'', \quad y''' = (F_x + y'F_y + z'F_z)y'' + (F_{y'} + KF_{z'})y''^2. \quad (4)$$

Kasner has discussed the innate projective character of dynamical trajectories. The concept of curvature trajectories is also projective. By Mehmke's theorem, it follows that the entire process of construction of curvature trajectories has projective meaning.

4. It is observed that the ∞^5 dynamical trajectories represented by (2) and the ∞^5 curvature trajectories defined by (4) are both special cases of systems of ∞^5 curves of the three-dimensional type (G) given by differential equations of the forms

$$z'' = K(x, y, z, y', z')y'', \quad y''' = G(x, y, z, y', z')y'' + H(x, y, z, y', z')y''^2, \quad (5)$$

where (K, G, H) are arbitrary functions in the five variables. Kasner has shown that such systems of ∞^5 curves of the three-dimensional type (G) are characterized geometrically by the following two properties.⁴

Property I. The ∞^1 curves which pass through a given lineal element E all have the same osculating plane.

Property II. If the osculating spheres are constructed at the lineal element E to the ∞^1 curves passing through E , the centers describe a straight line.

5. This resemblance between dynamical and curvature trajectories suggests the problem of determining all quintuply-infinite systems of curves in space which are simultaneously dynamical and curvature trajectories.

Kasner has completely solved this problem in the plane. *The appropriate families in the plane are exactly the trajectories of all central or parallel fields of force.* (See reference 2.)

Now we consider this problem in space. The proof of our result is long and complicated and will be published elsewhere. Our theorem is the following.

FUNDAMENTAL THEOREM. *The systems of ∞^b curves which are simultaneously dynamical and curvature trajectories are the dynamical trajectories of the following three distinct types of fields of forces:²*

- (I) *Those whose lines of force all lie in a pencil of planes.*
- (II) *Those whose lines of force are orthogonal to a family of ∞^2 circular helices, all of which possess the same axis and the same period.*
- (III) *Those of the central or parallel type.*

Thus the answer in space contains more types than in the plane. We find that each of these types is projectively invariant. The three distinct types of our Fundamental Theorem may be characterized in the following way. They are those families of dynamical trajectories whose ∞^b curves can be analyzed into a series of sets, each set containing ∞^4 curves, in such a way that one of the sets will generate the others by the simple process of multiplication of curvatures described above.

¹ Presented to the American Mathematical Society, November (1943).

² Kasner, "Dynamical Trajectories and Curvature Trajectories," *Bull. Amer. Math. Soc.*, **44**, 449-455 (1934).

³ Kasner, "Differential-Geometric Aspects of Dynamics," *Amer. Math. Soc. Colloquium Publications*, **3** (1913, 1934). Also see a series of papers in the *Trans. Amer. Math. Soc.*, **7-11** (1906-1910).

⁴ See the abstract of the paper presented before the American Mathematical Society in February, 1943: Kasner and De Ciccio, "Generalized Dynamical Trajectories in Space." Also see a forthcoming paper by De Ciccio, "Extensions of Certain Dynamical Theorems of Halphen and Kasner," *Bull. Amer. Math. Soc.*, (1943).

UNION-PRESERVING TRANSFORMATIONS OF DIFFERENTIAL-ELEMENTS

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1. Sophus Lie studied transformations from lineal-elements into lineal-elements. The contact group is obtained by requiring unions to be converted into unions. We generalize this theory by studying transformations from curvature-elements (x, y, y', y'') into lineal-elements (x, y, y') . An example of such a transformation arises in the ordinary theory of evolutes

$$X = x - \frac{y'}{y''} (1 + y'^2), \quad Y = y + \frac{1}{y''} (1 + y'^2), \quad Y' = -\frac{1}{y'} \quad (E)$$

We determine the general class of union-preserving transformations by means of a directrix equation. While Lie's standard directrix equation is of the form $\Omega(X, Y, x, y) = 0$, we find that our new directrix equation is of the form $\Omega(X, Y, x, y, y') = 0$. From our work, we deduce a generalized theory of evolutes and involutes which contains the standard evolute theory of Huygens and Bernoulli as a very special case. Finally the entire theory may be extended to transformations from differential-elements of order n into lineal-elements.

2. Any transformation from curvature-elements into lineal-elements may be given by the equations

$$X = \phi(x, y, p, q), \quad Y = \psi(x, y, p, q), \quad P = \chi(x, y, p, q), \quad (1)$$

where, of course, $p = y' = dy/dx$ and $q = y'' = dp/dx = d^2y/dx^2$.

A *union-preserving transformation* converts every union of curvature-elements into a union of lineal-elements. In general, tangent unions will not be converted into tangent unions. If two unions in the (x, y) -plane possess m as the order of contact, then the two corresponding unions in the (X, Y) -plane will have contact of order $(m - 1)$, at least.

Any transformation from curvature-elements into lineal-elements, not of the union-preserving type, carries exactly ∞^3 unions into unions. If a transformation converts more than ∞^3 unions into unions, then all ∞^∞ unions become unions, and therefore the transformation is of the union-preserving type.

The transformation (1) is union-preserving if and only if

$$\chi = \frac{\psi_x + p\psi_y + q\psi_p}{\phi_x + p\phi_y + q\phi_p} = \frac{\psi_q}{\phi_q}. \quad (2)$$

We wish to exclude from further consideration the following degenerate union-preserving transformations:

$$X = \text{const.}, \quad Y = \text{const.}, \quad P = \chi(x, y, p, q); \quad (3.1)$$

$$X = \phi(x, y, p, q), \quad Y = \text{const.}, \quad P = 0; \quad (3.2)$$

$$X = \text{const.}, \quad Y = \psi(x, y, p, q), \quad P = \infty; \quad (3.3)$$

$$X = \phi(x, y, p, q), \quad Y = \psi[\phi(x, y, p, q)], \quad P = \psi_\phi. \quad (3.4)$$

The first type carries not only every union, but all curvature-elements into a star (a point together with the ∞^1 directions through it). The second or third type carries every union and also all curvature-elements into a line parallel to the X - or Y -axis. The last type carries every union and also all curvature-elements into a single union.

From our further discussion, we wish not only to exclude the preceding degenerate transformations but also all those union-preserving transformations which convert every lineal-element (considered as a union of curvature-elements) into a star. Such transformations are defined by equations of the forms

$$X = \phi(x, y, p), \quad Y = \psi(x, y, p), \quad P = \frac{\psi_x + p\psi_y + q\psi_p}{\phi_x + p\phi_y + q\phi_p}. \quad (4)$$

3. By a *general union-preserving transformation* T , we shall mean any correspondence which sends every union into a union but which is not of the forms (3) or (4). For such a transformation T , it is noted that neither ϕ_q nor ψ_q vanishes. Therefore q can be eliminated from the first two of the equations (1), yielding the single relation

$$\Omega(X, Y, x, y, p) = 0. \quad (5)$$

We call this the *directrix equation* of the general union-preserving transformation T .

If we consider X and Y as parameters, the directrix equation defines *exactly* ∞^3 unions in the (x, y) -plane. On the other hand, if we regard x and y as parameters, the directrix equation can define at most ∞^1 unions in the (X, Y) -plane.

The directrix equation (5) defines one and only one general union-preserving transformation T and conversely.

A point in the (X, Y) -plane corresponds to ∞^1 unions in the (x, y) -plane which are defined by the directrix equation (5) where X and Y are constants. To find the second derivative q of any one of these unions, we

differentiate (5) totally with respect to x while keeping X and Y fixed. Thus we find

$$\Omega_x + p\Omega_y + q\Omega_p = 0. \quad (6)$$

Any lineal-element in the (x, y) -plane corresponds to a single union in the (X, Y) -plane. This is given by the directrix equation (5) where we regard (x, y, p) as constants. To find the slope of this single union, we differentiate (5) with respect to X where only Y is considered to depend upon X . Thus we have

$$\Omega_x + P\Omega_Y = 0. \quad (7)$$

Our general union-preserving transformation T is obtained by solving the equations (5), (6) and (7) for (X, Y, P) .

We note the complete analogy of our new theory to Lie's theory of contact transformations. In the Lie theory, any contact transformation is given by the directrix equation $\Omega(X, Y, x, y) = 0$, or else it is an extended point transformation. In our work, *any union-preserving transformation is given by the directrix equation (5), or else it is of the special form (4).*

4. Considered as a differential equation of first order in x, y, p , with X and Y as parameters, the directrix equation may be integrated, giving an equation of the form

$$\omega(X, Y, x, y, c) = 0, \quad (8)$$

where c is the constant of integration. We shall call this the *integrated form* of the directrix equation (5). In the (x, y) -plane, both (5) and (8) represent the same family of ∞^3 unions, whereas in the (X, Y) -plane, the ∞^3 unions corresponding to (x, y, p) are, in general, different from those corresponding to (x, y, c) .

Any union-preserving transformation from curvature-elements into lineal-elements is obtained by considering the osculating curves, of a given parameterized family of ∞^3 curves, to an arbitrary curve.

Suppose we are given ∞^3 curves in the finite form. We select two parameters X and Y and define them as a point of another plane. The finite form is then given by equation (8). Eliminating the constant c by differentiation, we obtain the directrix equation (5), from which the transformation T may be obtained. This process demonstrates that we are obtaining the curves of (8) which osculate (second order contact) any arbitrary curve.

From this point of view, it is seen that if Γ in the (X, Y) -plane is the corresponding union of γ in the (x, y) -plane, then Γ may be termed the *generalized evolute* of γ ; and γ is then termed a *generalized involute* of Γ with respect to the given family of ∞^3 parameterized unions defined by (5) or

(8). A single union has a single evolute, but to a single union there correspond ∞^1 involutes.

5. The entire preceding theory may be extended to transformations from differential-elements of order n into lineal-elements. Any such transformation may be defined by the equations

$$\begin{aligned} X &= \phi(x, y, p_1, \dots, p_n), & Y &= \psi(x, y, p_1, \dots, p_n), \\ P &= \chi(x, y, p_1, \dots, p_n), \end{aligned} \quad (9)$$

where, of course, $p_m = d^m y / dx^m$ for $m = 1, 2, \dots, n$.

Any transformation from differential-elements of order n into lineal-elements, not of the union-preserving type, carries exactly ∞^{n+1} unions into unions. If a transformation converts more than ∞^{n+1} unions into unions, then all unions become unions, and therefore the transformation is of the union-preserving type.

The transformation (9) is union-preserving if and only if

$$\chi = \frac{\psi_x + p_1 \psi_y + \dots + p_n \psi_{p_{n-1}}}{\phi_x + p_1 \phi_y + \dots + p_n \phi_{p_{n-1}}} = \frac{\psi_{p_n}}{\phi_{p_n}}. \quad (10)$$

By a general union-preserving transformation T , we shall mean any union-preserving transformation which does *not* carry every differential-element of order $(n - 1)$ (considered as a union of differential-elements of order n) into a star.

Any general union-preserving transformation T is defined by the directrix equation

$$\Omega(X, Y, x, y, p_1, \dots, p_{n-1}) = 0, \quad (11)$$

and conversely.

There are $\infty^{f(n+2)}$ general union-preserving transformations T .

Differentiate the directrix equation with respect to x while keeping X and Y constant. The result is

$$\Omega_x + p_1 \Omega_y + \dots + p_n \Omega_{p_{n-1}} = 0. \quad (12)$$

Also differentiate the directrix equation with respect to X where Y only depends on X . We find

$$\Omega_X + P \Omega_Y = 0. \quad (13)$$

Our general union-preserving transformation T is obtained by solving (11), (12) and (13) for (X, Y, P) .

Any general union-preserving transformation T from differential-elements of order n into lineal-elements is obtained by considering the osculating (contact of order n) curves of a given parameterized family of ∞^{n+1} curves, defined in the finite form by

$$\omega(X, Y, x, y, c_1, \dots, c_{n-1}) = 0, \quad (14)$$

where (X, Y) are the selected parameters and (c_1, \dots, c_{n-1}) are arbitrary parameters, to any arbitrary curve.

Of course, the preceding equation is the integrated form of the directrix equation (11).

6. *If a union-preserving transformation T from differential-elements into lineal-elements is such that any two unions which possess $n \geq 2$ as the order of contact are converted by T into two unions which have at least second order contact, then T must be a contact transformation of lineal-elements.*

From this result, we deduce the fact that, except for the extended Lie group of contact transformations of lineal-elements, there are no union-preserving transformations from differential-elements of order n into differential-elements of order m where $n \geq m > 1$. This includes as special cases the fundamental theorems of Lie and Backlund. *Therefore the only available union-preserving transformations (in the whole domain of differential-elements) are, firstly, the standard Lie contact group of lineal-elements and, secondly, our new set of union-preserving transformations from elements of order n (where n is 2 or more) into lineal-elements; or the extensions of these two types.*

In general, all the union-preserving transformations from differential-elements of order n into differential-elements of order m where $n \geq m$ are, firstly, if $n = m$, the extensions of order $(m - 1)$ of the Lie contact group of lineal-elements and, secondly, if $n > m$, the extensions of order $(m - 1)$ of our union-preserving transformations from differential-elements of order $(n - m + 1)$ into lineal-elements.

¹ Lie-Scheffers, "Berührungstransformationen" (1896).

² Kasner, "General Transformation Theory of Differential-Elements," *Amer. Jour. Math.*, **32**, 392-401 (1904).

³ Kasner and De Cicco, "Curvature Element Transformations Which Preserve Integrable Fields," *Proc. Nat. Acad. Sci.*, **25**, 104-111 (1939).

⁴ Kasner, "Lineal Element Transformations of Space for Which Normal Congruences of Curves Are Converted into Normal Congruences," *Duke Math. Journ.*, **5**, 72-83 (1939).

⁵ Kasner and De Cicco, "Transformation Theory of Integrable Double-Series of Lineal Elements," *Bull. Amer. Math. Soc.*, **46**, 93-100 (1940).

A FORMULA FOR THE STREAM FUNCTION OF CERTAIN FLOWS

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1. The potential and stream functions, ϕ and ψ , of an irrotational steady flow of a compressible perfect fluid satisfy a system of non-linear equations

$$\rho^{-1}\psi_v - \phi_x = 0, \quad \rho^{-1}\psi_x + \phi_v = 0,$$

where ρ is the density. (See [2.1].¹) Under the usual hypotheses $\rho = \rho(\phi_x^2 + \phi_v^2, k)$ is a function of $\phi_x^2 + \phi_v^2$ which depends upon a parameter k . This parameter enters in the equation of state, which we assume to have the form $p = a + c\rho^k$, a , c and k being constants.

Let $[v \exp(i\theta)]$ denote the velocity vector. If we introduce v and θ as independent variables then the equations connecting ϕ and ψ become linear. As Chaplygin showed the above system becomes

$$v^{-1}\rho\phi_\theta - \psi_v = 0, \quad \rho^{-1}v^{-1}(1 - M^2)\psi_\theta + \phi_v = 0. \quad [3.8], (1.1)$$

Here the Mach function, $M(v, k)$, and the density, $\rho(v, k)$, are given functions of v . Eliminating ϕ from (1.1) yields

$$S(\psi) \equiv \rho^{-2}(1 - M^2)\psi_{\theta\theta} + \rho^{-1}v[\rho^{-1}v\psi_v]_v = 0. \quad [2.6], (1.2)$$

In the case of an incompressible fluid, ψ is a harmonic function of θ and $\log v$. Taking the imaginary part of an analytic function of the complex variable $(\log v - i\theta)$ one obtains the stream function of a possible incompressible fluid flow. In § 2 and § 4 we shall define operators, \mathbf{P} , which transform analytic functions of one complex variable into solutions $\psi(v, \theta)$ of $S(\psi) = 0$. Thus we obtain a method for generating possible stream functions of compressible fluid flows. The computing of the corresponding stream function in the physical plane, i.e., of $\psi[v(x, y), \theta(x, y)]$, is reduced to quadratures. (See § 5 of [1].) We note, however, that the obtained flows do not always have a physical significance. (See § 4 of [1].)

2. Chaplygin was the first to introduce operators into compressible fluid theory.

Let $\Sigma B_n \tau^n \exp[i(2n\theta + \alpha_n)]$, $\tau = 1/2(k - 1)a_0^{-2}v^2$, $B_n \geq 0$, be the series development of an analytic function. Then the relation

$$\phi + i\psi = \Sigma B_n \tau^n Y_{n,0} Y_n [-(1 + n^{-1}\tau Y_n' Y_n^{-1})(1 - \tau)^{-\beta} \cos(2n\theta + \alpha_n) + i \sin(2n\theta + \alpha_n)] \quad (2.1)$$

yields a pair, ϕ and ψ , satisfying the system (1.1). Here $\beta = (k - 1)^{-1}$, $Y_n = F(a_n, b_n, 2n + 1, \tau)$, where F is the hypergeometric series, and $a_0, a_n, b_n, Y_{n,0}$ suitably chosen constants.² We obtain another operator by the following procedure: By $[\theta + iH]^{[n]}$ we understand the following:

$$\sum_{\kappa=0}^n C_{n,\kappa} \theta^{n-\kappa} (iH)^\kappa, \text{ but then replace each } \theta^{n-\kappa} H^\kappa \text{ by}$$

$$\kappa! \theta^{n-\kappa} \int_0^H \dots \int_0^{H_{s+1}} dH_{s+1} \int_0^{H_{s+1}} l(H_s) dH_s \int_0^{H_s} dH_{s-1} \int_0^{H_{s-1}} l(H_{s-2}) dH_{s-2} \int_0^{H_{s-2}} \dots$$

κ integrations

$$l(H) = \rho^{-2}(1 - M^2). \quad [8.21], (2.2)$$

Here H is given by the relation $dH/dv = \rho/v$. The $C_{n,\kappa}$ are binomial coefficients.

$$\phi + i\psi = c[\theta + iH]^{[n]} \quad [8.22], (2.3)$$

yields a pair of functions satisfying (1.1).³ Here c is a real or purely imaginary constant. The factor $l(H_s)$ is to precede dH_s for even s when c is real, and for odd s when c is pure imaginary. The proof of the above statement follows immediately by substituting ϕ and ψ which are introduced in (2.3) into [8.18] of [1]. Thus if $\sum(\alpha_n + i\beta_n)\zeta^n$ is the series development of an analytic function,

$$\phi + i\psi = \sum(\alpha_n + i\beta_n)[\theta + iH]^{[n]} \quad (2.4)$$

yields a pair of functions satisfying (1.1).

An analytic function is representable by its series development only in the largest circle in which it is regular. Since the operators (2.1) and (2.4) depend upon the choice of the origin the usual method of analytic continuation does not preserve the connection between g and $\psi = P(g)$. Thus by (2.1) and (2.4) the functions ϕ and ψ are defined locally. The continuation of ϕ and ψ is a separate question which often presents still unsolved problems of considerable difficulty. On the other hand, in many instances in order to obtain a compressible fluid flow similar to an incompressible one, it is necessary to determine the stream function $\psi = P(g)$ in the *whole* (not necessarily schlicht) domain \mathfrak{M} in which g is defined. This is the reason why, in addition to the operators (2.1) and (2.4), we introduce in § 4 a new operator P which defines the solution $P(g)$ of (1.2) in the whole domain \mathfrak{M} .

3. In order to define the above-mentioned operator we transform S into the canonical form (3.3). We assume in the following that⁴ $-1 \leq k < 1$, and that the flow is subsonic. By the relation

$$2\lambda = 2\gamma \arctan [\gamma^{-1}(1 - M^2)^{1/2}] + \log [(1 - (1 - M^2)^{1/2})/(1 + (1 - M^2)^{1/2})], \quad (3.1)$$

where $M = v[a_0^2 - 1/2(k - 1)v^2]^{-1/2}$ is the Mach function (see [2.6]), $\gamma = [(1 + k)^{1/2}(1 - k)^{-1/2}]$ and a_0 is a constant, we introduce the variable λ .

If v varies from 0 to the velocity of sound, M varies from 0 to 1, λ varies from $-\infty$ to 0 and T varies from 1 to 0.

LEMMA: Let $M \equiv \mu(2\lambda)$ be the function inverse to (3.1) and let $T(2\lambda) = [1 - \mu^2(2\lambda)]^{1/2}$. Then for $2\lambda < 0$,

$$\begin{aligned} T(2\lambda) = 1 - X - 1/2(2k + 1)X^2 - 1/4(4k^2 + 6k + 3)X^3 - \\ 1/24(24k^3 + 68k^2 + 76k + 29)X^4 - 1/48(48k^4 + 212k^3 + 392k^2 + \\ 328k + 103)X^5 - 1/480(480k^5 + 2976k^4 + 7968k^3 + 10,788k^2 + \\ 7266k + 1935)X^6 + 1/2880(2880k^6 + 23472k^5 + 84232k^4 + \\ 162124k^3 + 173940k^2 + 98086k + 22675)X^7 + \dots \end{aligned} \quad (3.2)$$

where $X = 2 \exp [2\lambda - \gamma(\pi - 2 \arctan \gamma)]$. The proof of the convergence of (3.2) follows from the fact that $T[\log (X/2) + \gamma(\pi - 2 \arctan \gamma)]$ considered as an analytic function of a complex variable X is regular in the circle $|X| < 2 \exp [-\gamma(\pi - 2 \arctan \gamma)]$. By introducing the variables λ and θ , (1.2) becomes

$$\begin{aligned} L_0(\psi) \equiv 1/4 \Delta \psi + N(2\lambda)\psi_\lambda = 0, \quad [6.6], \quad (3.3) \\ N(2\lambda) = -1/8(k + 1) [1 - T^2(2\lambda)][T(2\lambda)]^{-3}. \end{aligned}$$

4. THEOREM. Let ψ^* satisfy the equation

$$L(\psi^*) \equiv 1/4 \Delta \psi^* + F(2\lambda)\psi^* = 0, \quad (4.1)$$

where $F(2\lambda)$ is defined for $\lambda \leq \alpha$, $\alpha \geq 0$, and possesses the property that

$$|d^k F(2\lambda)/d\lambda^k| \leq c\Gamma(\kappa + 1)(\alpha - \lambda)^{\kappa+2} \text{ for } \kappa = 0, 1, 2, \dots \text{ and } \lambda < \alpha, \quad (4.2)$$

$c < \infty$ being a suitably chosen constant. Let $g(\zeta)$ be an analytic function regular in a domain \mathfrak{B} which contains the origin. Then

$$\begin{aligned} \psi^*(\lambda, \theta) = \\ \operatorname{Im} [g(\zeta) + \sum_{n=1}^{\infty} 2^{-2n} \Gamma(2n + 1) [\Gamma(n + 1)]^{-2} Q^{(n)}(2\lambda) \underbrace{\int_0^\zeta \dots \int_0^\zeta g(\zeta) d\zeta^n}_{n \text{ times}}] \end{aligned} \quad (4.3)$$

will be a solution of (4.1), and (4.3) will represent it in every simply connected domain which contains the origin and lies in $\mathfrak{B} \cap \mathfrak{G}$. \mathfrak{G} denotes the domain $[\lambda^2 + \theta^2 < 4(\lambda - \alpha)^2, \lambda < \alpha]$.

The functions $Q^{(n)}(2\lambda)$ are defined by the recurrence formula:

$$(2n + 1)Q_{\lambda\lambda}^{(n+1)} + Q_{\lambda\lambda}^{(n)} + 4FQ^{(n)} = 0, \quad Q_{\lambda}^{(1)} = -4F, \quad Q^{(n)}(a) = 0, \\ a < 0.$$

Proof. If the series $1 + t\zeta^{1/2}\sum_{n=1}^{\infty}(t\zeta^{1/2})^{2n-1}Q_{2\lambda}^{(n)}$ which we shall denote by E , converges, it represents a solution of the equation (1.2) of [3].⁵ Then by Theorem 1 of [3] the integral $\int_{-1}^{+1} E(\zeta, \bar{\zeta}, t) f[1/2\zeta(1-t^2)] dt / (1-t^2)^{1/2}$, where $f(\eta)$ is an arbitrary analytic function of a complex variable η , will be a particular solution of (4.1). Writing $g(\zeta) = \int_{-1}^{+1} f[1/2\zeta(1-t^2)] dt / (1-t^2)^{1/2}$ we obtain (4.3). The proof of the convergence of the series $\sum t^{2n}\zeta^n Q^{(n)}(\zeta + \bar{\zeta})$ is similar to that of Theorem 2 of [3]. Taking the quantities $\tilde{Q}_{\lambda}^{(n+1)}$ given by $(2n+1)\tilde{Q}_{\lambda}^{(n+1)} = \tilde{Q}_{\lambda\lambda}^{(n)} + c(\alpha - \lambda)^{-2}[\int_a^{\lambda} \tilde{Q}_{\lambda}^{(n)} d\lambda + n^{-1}c^{(n)}(\alpha - a)^{-n}]$, where the $c^{(n)}$ are positive constants, as majorants for $Q_{\lambda}^{(n+1)}$, one obtains $\tilde{Q}_{\lambda}^{(n)} = c^{(n)}(\alpha - \lambda)^{-n-1}$, $\lim_{n \rightarrow \infty} c^{(n)} = 1/2$.

If we substitute

$$\psi(\lambda, \theta) = [\exp(-\int_{-\infty}^{2\lambda} N(2\lambda) d(2\lambda))] \psi^*(\lambda, \theta) \quad (4.4)$$

in the equation (3.3), the latter becomes (4.1). Under physical conditions which usually occur, the obtained coefficient of ψ^* in (4.1) satisfies the conditions (4.2) in some cases. In other cases, it is possible to approximate it by a function satisfying (4.2).

Combining (4.4) and (4.3) we obtain an operator⁶ P possessing the property mentioned at the end of § 2.

Remark. We note that:

$$L(2\lambda) = -\int_{-\infty}^{2\lambda} N(2\lambda) d(2\lambda) = [-1/2 \log T + 1/2(1-k)^{-1} \log(\gamma^2 + T^2)]_{T=1}^{T(2\lambda)}$$

$$Q^{(1)}(2\lambda) = [-5/24(k+1)T^{-3} + 1/4kT^{-1} + 1/8(1-3k)(1+k)(1-k)^{-1}T - (1-k)^{-1}(1-k^2)^{-1/2} \arctan(\gamma/T)]_{T=1}^{T(2\lambda)}.$$

5. Let $\mathbf{q} = v \exp(i\theta)$ denote the velocity vector of a flow. A flow $\mathfrak{F}(\mathfrak{B})$ is said to be of the type D_n if the boundary of the domain \mathfrak{B} in which \mathfrak{F} is defined consists of a segment of the negative real axis $(-\infty, a)$ and of $2n$ segments S_{κ} , $\kappa = 1, \dots, 2n$, such that along every $S_{2\kappa-1}$, $\kappa = 1, 2, \dots, n$, $\theta = \theta_{\kappa} = \text{const.}$, $\pi/2 \geq \theta_1 > \theta_2 > \dots > \theta_n > 0$, and along every $S_{2\kappa}$, $v = v_{\kappa} = \text{const.}$, $v_{\kappa-1} < v_{\kappa}$. The $S_{2\kappa-1}$ are segments of straight lines, while the $S_{2\kappa}$ are so-called "free boundaries." The images \mathfrak{U} and \mathfrak{P} of \mathfrak{B} in the $v\theta$ plane and in the $\lambda\theta$ plane, respectively, are domains bounded by segments of the lines $v = \text{const.}$, $\theta = \text{const.}$ and $\lambda = \text{const.}$, $\theta = \text{const.}$, respectively. The complex potential $w(\lambda, \theta)$ of the flow \mathfrak{F} of the type D_n is the function which maps \mathfrak{P} into the upper half plane, \mathfrak{U} .

Using the theory of orthogonal functions⁷ one can obtain an explicit formula for the function $h(\zeta)$ which conformally maps a domain \mathfrak{P} into \mathfrak{U} . We set

$$\phi'_{m+1}(\eta) = \begin{vmatrix} F_{00} & \dots & F_{0m-1} & 1 \\ \cdot & \dots & \cdot & \cdot \\ F_{m0} & \dots & F_{mm-1} & \eta^m \end{vmatrix} \cdot \left[\begin{vmatrix} F_{00} & \dots & F_{0m-1} & \cdot \\ \cdot & \dots & \cdot & \cdot \\ F_{m-10} & \dots & F_{m-1m-1} & \cdot \end{vmatrix} \cdot \begin{vmatrix} F_{00} & \dots & F_{0m} \\ \cdot & \dots & \cdot \\ F_{m0} & \dots & F_{mm} \end{vmatrix} \right]^{-1/2} \quad (5.1)$$

where

$F_{mp} = \sum_{k=0}^n (m+p+2)^{-1} v_k^{m+p+2} T_{mk}$, $T_{mp} = -i(m-p)^{-1} [\exp(\theta_k(m-p)) - \exp(\theta_{k+1}(m-p))]$ if $m \neq p$ and $T_{mm} = (\theta_k - \theta_{k+1})$. See [6] p. S II 1. If we write $N(\eta, \bar{t}) = \sum_{m=0}^{\infty} \phi_{m+1}(\eta) \phi'_{m+1}(\bar{t})$, $\phi_{m+1}(\eta) = \int_0^{\eta} \phi'_{m+1}(\eta) d\eta$, then

$$h(\zeta) = -i \frac{\sqrt{\pi} \exp(-\zeta + i\beta) N_{\zeta}(\exp \zeta, \bar{t}) - [N(t, \bar{t})]^{1/2}}{\sqrt{\pi} \exp(-\zeta + i\beta) N_{\zeta}(\exp \zeta, \bar{t}) - N(t, \bar{t})}^{1/2}. \quad (5.2)$$

See § X.1-§ X.3 of [6]. Here β and t are constants which have to be chosen in a suitable way.

For $k = -1$, $\psi = \text{Im}(h)$ represents the stream function of a flow of the type D_n . (See footnote 6.) For other values of k , $P(h)$ will represent the stream function of a flow of a compressible fluid, the boundary of which can be decomposed into $(2n+1)$ segments S_k^* such that along S_{2k-1}^* , θ is nearly constant, and along S_{2k}^* , v is nearly constant.⁸

¹ The reader will find a detailed derivation of some results and of some formulae used here, in [1], Bergman, "The Hodograph Method in the Theory of Compressible Fluid" (Supplement to "Fluid Dynamics" by v. Mises and Friedrichs), Publication of Brown University, 1942. In order to facilitate the reading we indicate in the brackets the number under which the corresponding formula or section is listed in this work.

A typewritten enlarged copy of this Note with detailed computations is available at Brown University Library.

² See [2], Chaplygin, "Gas Jets," *Memoirs Univ. Moscow, Ph.-Math. Section*, 21, 1-121 (1904).

In connection with the introduction of the hodograph plane the investigation of solutions for which $\partial(\phi, \psi)/\partial(\log v, \theta)$ is identically 0 or ∞ would be of interest.

³ Dr. Bers and Dr. Gelbart in a joint investigation have obtained independently of the author, these same particular solutions. In their work which appears in the *Quart. Appl. Math.*, 1 (1943), this result follows as a consequence of their approach to more general partial differential equations by complex variable methods. Concerning the connection between the functions (2.3) and (2.1) I refer to this paper.

I note further that Dr. Vazsonyi by a different method obtained particular solutions $\psi(H, \theta)$ of the following form: $\sum_{k=0}^n \theta^{n-k} V_{nk}(H)$ where $V_{nk}(H)$ are polynomials in H .

⁴ The case $k > 1$ can be developed similarly. In this case, instead of (3.1), we use

$$2\lambda = \log \left[\frac{1 + (1 - M^2)^{1/2}}{1 - (1 - M^2)^{1/2}} \left(\frac{1 + h(1 - M^2)^{1/2}}{1 - h(1 - M^2)^{1/2}} \right)^{1/2} \right], \quad h = \left(\frac{k-1}{k+1} \right)^{1/2}.$$

(See [6.5] of [1].)

⁵ See [3], Bergman, *Matem. Sbornik (Recueil Math.)*, n. s. 2(44), 1169-1198 (1937).

⁶ In the case where $k = -1$, $L = Q^{(n)} = 0$, i.e., $\psi = P(g)$ is a harmonic function and the operation $P(g)$ consists in the taking of the imaginary part. This fact was established by Chaplygin, and it forms the basis of one of his methods and of that of v. Kármán-Tsien. See [4] v. Kármán, *Journ. of Aer. Sci.*, 8, 337-356 (1941), and [5] Tsien, *Ibid.*, 6, 399-407 (1939).

⁷ See [6] Bergman, "Partial Differential Equations," Publication of Brown University, 1941, VI-XI.

⁸ We note that, using the theory of integral equations, one can prove the existence of the stream function of a flow of the type D_n , and that many flows which are of interest in fluid dynamics may be approximated by flows of the type D_n .

CORRECTION TO "ON CERTAIN NON-LINEAR DIFFERENTIAL EQUATIONS OF THE SECOND ORDER"

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In the above-cited paper¹ several properties of a certain class, C , of differential equations were described. The author claimed that the equation

$$\ddot{x} + f(x)\dot{x} + g(x) = e(t), \quad (1)$$

with $e(t)$ of period L , was in class C subject to several conditions, the main one being $f(x) \geq a > 0$.

To describe class C we observe that any point $P_0(x_0, \dot{x}_0)$ in the (x, \dot{x}) plane is transformed into a point $P_1(x_1, \dot{x}_1)$ by taking the solution $x(t)$ of (1) for which $x(0) = x_0$ and $\dot{x}(0) = \dot{x}_0$, and setting $x_1 = x(L)$ and $\dot{x}_1 = \dot{x}(L)$. This transformation of P_0 into P_1 we denote by T . Thus $TP_0 = P_1$. The class C contains those equations of (1) whose maximum invariant finite domain under T is a dendrite. With $f(x)$ positive, the maximum invariant finite domain of (1) can be shown to have plane measure zero and its complement is an open continuum which is simply connected if the point at infinity is adjoined to the (x, \dot{x}) plane. But such a domain can deviate so considerably from a dendrite that (1) with $f(x) \geq a > 0$ falls outside of the scope of the present proof. The author therefore withdraws his statement that (1) with $f(x) \geq a > 0$ is in class C .

¹ Levinson, N., "On Certain Non-Linear Differential Equations of the Second Order," *Proc. Nat. Acad. Sci.*, 29, 222 (July, 1943).

STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. II. *ASPERGILLUS FLAVUS-ORYZAE* GROUP*

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The production of antibiotic substances by microorganisms is not a property characteristic of specific groups of organisms or even of given species within such groups, but of a few selected strains within a given species. This has been demonstrated, in the case of the fungi, for *Penicillium notatum*,⁵ *Aspergillus fumigatus*⁶ and *Aspergillus clavatus*.⁷

When several strains of the same organism were compared it was found that differences in the composition of the medium and in the environmental conditions greatly influence not only the yield of the antibiotic substance but also its nature or chemical composition. It is sufficient to cite the effect of corn steep upon the production of the active substance by *P. notatum*:⁶ in the case of one strain (No. O), an active producer of true penicillin, the yield was increased but the concentration of the *Escherichia coli* factor⁹ was reduced; in the case of another strain (No. W), an active producer of the *E. coli* factor, no favorable effect was exerted upon the production of penicillin, although the *E. coli* factor was depressed. Differences in the production of clavacin by a series of cultures of *A. clavatus* were shown to be due, partly at least, to the inactivation of the active substance by the alkalinity of the medium produced by some of the strains.⁷

One would expect that similar variations in the physiology of the organisms would also hold true for other groups of antagonistic fungi. *Aspergillus flavus* represents such a group and is widely distributed in nature. The ability of members of this group to produce an antibacterial substance has been demonstrated by White⁸ and by Glister.³ According to White, *A. flavus* is capable of growing in synthetic media, but it requires complex organic forms of nitrogen, such as tryptone, peptone and corn steep, for the production of an antibiotic substance, designated as aspergillic acid. This isolated substance had about the same effect against gram-positive as against gram-negative bacteria; on the other hand, the culture filtrate showed greater specificity towards the first. This suggested the possibility that another substance may have been present in the filtrate and was not extracted with the aspergillic acid. Glister also emphasized the wide range of antibacterial activities of the culture filtrate of *A. flavus*.

Jones, Rake and Hamre² isolated from White's culture different strains that showed definite variation in the production of the antibiotic substance; however, no difference could be observed in the nature of the bacteriostatic

spectrum between the metabolic filtrates of young and old cultures, on the one hand, and of the isolated aspergillic acid, on the other. Bush and Goth¹ obtained from a strain of *A. flavus* another substance, designated as flavicin, that was very similar to penicillin in the nature of its bacteriostatic spectrum, low toxicity to animals and activity *in vivo*. McKee and MacPhillamy⁴ also demonstrated, by chemical, biological and chemotherapeutic tests, that the second factor produced by *A. flavus* is penicillin.

In all the above studies, the cultures of *A. flavus* were apparently chance air contaminants. In a survey carried out in our laboratories⁵ on the occurrence of antagonistic fungi in nature, only one strain belonging to the *A. flavus* group was isolated. The culture filtrate of this organism showed only limited antibacterial activity, largely directed against certain gram-positive bacteria. On further study, however, it was found that when the composition of the medium was modified by the addition of complex organic materials and when conditions of growth were changed from stationary to submerged, there was a marked improvement in the production of the antibiotic substance by this organism.

Because of the peculiar behavior of the particular strain of *A. flavus*, it was decided to make a more comprehensive study of the production of antibiotic substances by this group of organisms. Six different cultures were obtained from as many sources. They are listed here as follows:

No. 26 represents the culture of Glister, who isolated it in England.

No. 27 was isolated by White.

No. 31 is a culture of *A. flavus* originally obtained from C. Thom in Washington and kept for many years in the N. J. Culture Collection.

No. 134 was isolated by Pvt. A. Schatz, while stationed at the Miami Beach Military Hospital, in April, 1943, from a *Meningococcus* blood agar plate.

No. 135 was received from Miss M. B. Morrow of Texas, who isolated it from a soil in Guatemala.

No. 137 was isolated 2 years previously in the aforementioned survey of the occurrence of antagonistic fungi in nature.

All these cultures represented typical forms of *A. flavus*. They showed minor differences, however; No. 27 tended to produce yellow spores and turned green only later, if at all; No. 135 was the only strain that produced sclerotia abundantly.

In addition to these six cultures of *A. flavus*, five cultures of a closely related group of fungi, *A. oryzae*, were selected from our culture collection and tested for their ability to produce antibiotic substances. These cultures were obtained at various times from different laboratories and were known to be active producers of diastatic enzymes. With one exception, they showed no antibacterial activity whether grown in a stationary or in

TABLE 1

PRODUCTION OF ANTIBIOTIC SUBSTANCES BY DIFFERENT STRAINS OF *A. flavus*
Corn Steep Medium, Submerged Culture^a

STRAIN NO.	ACTIVITY BY PLATE METHOD, UNITS ^b				ACTIVITY BY CUP METHOD, ^c ZONE OF INHIBITION, MM.	PH OF MEDIUM
	<i>E. coli</i>	<i>B. mycoides</i>	<i>B. subtilis</i>	<i>S. lutea</i>		
26	3	10	75	100	20	7.9
27	0	0	0	0	14	7.8
31	0	0	75	>300	30	8.0
134	0	0	100	>300	29	7.6
135	0	5	75	100	26	7.9
137	0	0	150	>300	26	...

^a Five days' incubation at 30°C.

^b Units of activity = ratio between volume of medium and smallest volume of culture filtrate showing inhibition of test organism.

^c Spore suspension of standard *B. subtilis* culture used; undiluted culture filtrate placed in cups.

TABLE 2

INFLUENCE OF COMPOSITION OF MEDIUM AND CONDITIONS OF GROWTH UPON THE
PRODUCTION OF AN ANTIBIOTIC SUBSTANCE BY DIFFERENT STRAINS OF *A. flavus*

NATURE OF MEDIUM STRAIN NO.	CORN STEEP				TRYPTONE-GLUCOSE	
	SUBMERGED, 5 DAYS		STATIONARY, 8 DAYS		SUBMERGED, 8 DAYS	
	<i>B. subtilis</i>	<i>S. lutea</i>	<i>B. subtilis</i>	<i>S. lutea</i>	<i>B. subtilis</i>	<i>S. lutea</i>
26	75	500	0	0	15	30
27	0	0	0	0	10	10
31	75	200	0	0	0	0
134	25	100	5 ^a	5	10	10
135	100	500	30	30	30	>100
137	200	1000	0 ^a	0	10	10

^a In another experiment, 6-day old stationary cultures gave for No. 134—30 *B. subtilis* and 25 *S. lutea* units, and for No. 137—25 and 75 units, respectively.

TABLE 3

COURSE OF FORMATION OF ANTIBIOTIC SUBSTANCES BY 2 STRAINS OF *A. flavus*
Corn Steep Medium, Submerged Culture

INCUBATION, DAYS	STRAIN NO.	ACTIVITY, UNITS		CUP METHOD, MM.	REACTION, PH
		<i>B. subtilis</i>	<i>S. lutea</i>		
2	135	0	30	12.0	5.8
3	135	5	30	16.0	7.0
4	135	25	25	22.5	7.7
5	135	25	100	18.0	...
2	137	5	100	18.5	6.1
3	137	50	1000	20.5	7.2
4	137	75	750	26.5	7.3
5	137	100	750	26.0	...

a submerged condition, in synthetic or in organic media. Even the exceptional culture gave only low antibiotic activity, namely, 10 *Sarcina lutea* units per 1 ml. of filtrate, when grown under the most favorable conditions.

When grown on the ordinary Czapek-Dox medium, whether in a stationary or in a submerged state, the various cultures of *A. flavus* produced only little antibiotic activity. This phenomenon has already been reported by White.⁸ When a corn steep medium¹⁰ was used, some of the strains, especially when grown in a submerged and aerated condition, produced a highly active filtrate. This is brought out in tables 1 and 2. Even these culture filtrates showed very little activity, however, against *E. coli* and *Bacillus mycoides*, occasionally, some of the strains showed limited activity against either or both of these two test bacteria. The course of production of the antibiotic substance by two of the most active strains, 135 and 137, is shown in table 3.

Since aspergillic acid acts more or less alike against the different types of bacteria, both gram-positive and gram-negative forms, one must conclude from the results presented in these tables that the antibiotic substance produced under submerged conditions by the different strains of *A. flavus* is not entirely comparable to the true aspergillic acid, found by White and others, but represents a type of compound comparable to the flavicin of Bush and Goth.¹

At least three factors are involved in the production of the antibiotic substances by *A. flavus*, namely: (1) the specific nature of the strain of organism used, (2) the composition of the medium in which it is grown, (3) the conditions of growth, especially the aeration of the cultures. It is interesting to note that in submerged culture, No. 137 proved to be the most active strain when grown in the corn steep medium; however, in the submerged tryptone-glucose medium and in the stationary corn steep medium, No. 135 proved to be most active.

The culture filtrates of the different strains of *A. flavus* grown in the submerged corn steep medium were acidified and extracted with ether. The extract was evaporated, taken up in alcohol, in which it dissolved readily, and tested for antibiotic activity. On the basis of one gram of dry material, the following units were obtained: 175,000 *B. subtilis*, 1,000,000 *S. lutea*, 50,000 *B. mycoides* and 5000 *E. coli*. Whereas the culture filtrate had no activity against *E. coli* and little activity against *B. mycoides*, the extracted substance had a definite antibiotic action against the *E. coli* and especially against the *B. mycoides*. Quantitatively, the fraction thus extracted represented only a small part of the activity of the culture filtrate (about 10 per cent). Comparison of the bacteriostatic spectrum of the original culture filtrate with that of the isolated material clarifies one point, namely, that the isolated material behaves in a manner

similar to aspergillic acid, whereas the spectrum of the culture filtrate is similar to penicillin and is, therefore, comparable to flavicin.

In order to compare these data with those obtained for pure aspergillic acid,¹¹ a sample of the latter was tested and found to contain 500,000 *B. subtilis*, 300,000 *S. lutea*, 100,000 *B. mycoides* and 33,000 *E. coli* units; this type of spectrum is very similar to that obtained for the fraction isolated from the *A. flavus* submerged cultures by the above method. One must conclude, therefore, that *A. flavus* in submerged culture produces two substances, one that comprises a smaller fraction and is comparable to aspergillic acid, and another that makes up the major part of the active material in the culture and is comparable in its activity to penicillin and may be considered identical with flavicin.

In order to demonstrate further the identity of the bacteriostatic spectrum of the two organisms, the results of the following experiment may be reported here. Two of the most active strains of *A. flavus* (No. 137) and of *P. notatum* (No. 50) were grown in the corn steep medium in a submerged condition at 28°C., the cultures being shaken continuously, to provide aeration and agitation. At different intervals of time, some of the flasks were removed and analyzed (table 4). The activity of the *A. flavus* was slow at first, but reached an optimum in 5 days, whereas that of *P. notatum* rose more rapidly; the reaction of culture filtrate of the first never became acid, but remained at pH 7.5 to 7.8 for some time, whereas that of the second became at first acid, then changed to alkaline, reaching pH 8.5 in 5 days. Detailed results of the tests for this period are given in table 5. These results show in a most striking manner that the antibacterial nature of the antibiotic substance of *A. flavus* is exactly the same as that of *P. notatum*, as represented by the bacteriostatic spectra of the two organisms; any differences reported are quantitative rather than qualitative in nature.

The crude extract was rather toxic for mice, but upon further purification the toxicity was reduced to such an extent that a dose level of 400 mgm. per kgm. of the final product was tolerated. Although this preparation appears to be somewhat more toxic than penicillin, the fact that purification tends to reduce the toxicity suggests that upon the complete removal of the toxic principle, the order of toxicity of this agent will be similar to that of penicillin.¹²

It has already been established that different antagonistic organisms produce more than one antibiotic factor. *P. notatum* produces penicillin and notatin; *A. fumigatus* produces fumigatin and fumigacin, etc. To these must now be added *A. flavus*, which produces aspergillic acid and a substance comparable to penicillin and designated¹ as flavicin. To what extent antibiotic substances produced by different groups of microorganisms represent the same type of chemical compound is a matter for

TABLE 4

COURSE OF PRODUCTION OF ANTIBACTERIAL SUBSTANCES BY *A. flavus* AND *P. notatum*
Submerged Growth, 28°C. Incubation

INCUBA- TION, DAYS	PH OF CULTURE ^a	<i>A. flavus</i> UNITS OF ACTIVITY		PH OF CULTURE ^a	<i>P. notatum</i> UNITS OF ACTIVITY	
		<i>S. aureus</i>	<i>B. subtilis</i>		<i>S. aureus</i>	<i>B. subtilis</i>
2	7.8	Trace	5	6.3	200	250
3	7.5	30	50	6.7	200	300
5	7.8	200	200	8.5	400	500
7	8.1	150	150	8.7	100	150
9	8.4	100	150	9.0	20	50

^a Original reaction of medium after sterilization was 6.2.

TABLE 5

BACTERIOSTATIC SPECTRUM OF THE ANTIBIOTIC SUBSTANCES PRODUCED BY *A. flavus* AND
P. notatum

Units of Activity, 5-Day-Old Culture Filtrate

TEST ORGANISM	<i>A. flavus</i>	<i>P. notatum</i>
<i>Staphylococcus aureus</i> H	200	400
<i>Staphylococcus aureus</i> D	100	300
<i>Sarcina lutea</i>	500	1000
<i>B. subtilis</i> O	200	500
<i>B. subtilis</i> 243*	15	20
<i>B. mycoides</i>	<10	<10
<i>B. cereus</i>	<10	<10
<i>Shigella gallinarum</i>	<10	<10

* This strain is inaccurately designated as *B. subtilis*; it is more closely related to *B. mycoides*.

further study. Too little is yet known of the chemistry of these compounds to justify generalizations. The limited information available warrants, however, the suggestion that although those antibiotic substances that are already known vary greatly in chemical composition, certain well-defined types of compounds may be produced by different organisms. This may hold true in the production of penicillin, namely, by members of the genus *Penicillium* (*P. notatum* and *P. chrysogenum*) and by members of the genus *Aspergillus* (*A. flavus*).

Summary.—A study was made of the production of antibiotic substances by six different strains of *A. flavus* and of five strains of *A. oryzae*. The *A. oryzae* strains had little or no antibacterial activity. The activity of the culture filtrate of the *A. flavus* strains was found to depend on the nature of the strain, the chemical composition of the medium and the conditions of growth, especially aeration and agitation.

The bacteriostatic spectra of the culture filtrate of *A. flavus* and of the active substances isolated from it tended to prove that the antibiotic activity of this organism is due to two distinct factors, namely, (a) asper-

gillic acid, which is active against gram-negative and gram-positive bacteria; (b) flavicin, comparable if not identical with penicillin, which is active largely against gram-positive bacteria.

One of the cultures of *A. flavus*, isolated in a survey of the occurrence of antagonistic fungi in nature, produced under submerged conditions of growth enough flavicin to compare favorably with the production of penicillin by the best strains of *P. notatum* grown under the same conditions. The bacteriostatic spectra of the two preparations were identical.

* Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Soil Microbiology.

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⁹ This factor has been recently described as penatin, notatin and penicillin B.

¹⁰ The corn steep medium used in these studies is a modification of a medium previously employed.⁸

¹¹ The authors are indebted to The Squibb Institute for the sample of crystalline aspergillic acid.

¹² The authors are indebted to Dr. H. Robinson of the Merck Institute for making the toxicity tests.

COMPETITION BETWEEN FREE AND COMBINED NITROGEN IN NUTRITION OF AZOTOBACTER*

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A thorough knowledge of the effect of various forms of combined nitrogen on the assimilation of molecular nitrogen may be highly suggestive regarding their rôle as possible intermediates in biological nitrogen fixation. The stable isotope of nitrogen, N^{15} , offers a convenient means for following the utilization of fixed and molecular nitrogen simultaneously. If a culture of nitrogen-fixing organisms is supplied simultaneously with molecular nitrogen enriched with N^{15} and combined nitrogen compounds containing the normal ratio of the nitrogen isotopes, the final level of N^{15} in the cells measures accurately what portion of their nitrogen they derived from molecular N_2 and what portion from the combined nitrogen supplied.

Many of the early tests to determine the effect of combined nitrogen on nitrogen fixation were conducted with cultures of questionable purity and under conditions far from optimum for growth of the organism. Burk and Lineweaver,¹ in reporting the effect of fixed nitrogen compounds as measured in short-time micro-respiration experiments, emphasized the erroneous conclusions which may arise from experiments in which the growth of the organism extends over a period of weeks. Burk² has lately called attention to the scarcity of recent work on the utilization of fixed nitrogen compounds by *Azotobacter* and has stated that, "Older work needs to be repeated with adequate supplies of trace elements (iron, molybdenum, vanadium, tungsten, etc.)."

Experimental.—Burk's¹ nitrogen-free, 2 per cent sucrose medium is inoculated with a 24-hour culture (1 drop per 10 ml.) of the free-living, aerobic, nitrogen-fixing organism, *Azotobacter vinelandii*. The inoculated medium is shaken to distribute the organisms, and 25-ml. portions are pipetted into sterile culture flasks.³ The normal nitrogen compounds are added in 1 ml. of sterile, neutral solution, and 1 ml. of sterile water is added to the nitrogen-free controls. Sterile 50 per cent KOH (1 ml.) is placed in the inset cup for absorption of CO_2 . The flasks are plugged with sterile cotton and placed on a manifold in a 30°C. constant temperature bath. The system is evacuated, and O_2 and N_2 are added to the desired pressures; in all experiments a pO_2 of 0.2 atmosphere and a pN_2 of 0.2 atmosphere were employed leaving a 0.6 atmosphere vacuum. Oxygen is supplied from a 2-liter reservoir during the experiment. After a suitable period of

growth, as judged by the amount of oxygen used by the culture, the gas is recovered, the culture is removed from the flask and the cells are washed by centrifugation. The supernatant from the first centrifugation of the cells is analyzed to check that an excess of combined nitrogen remains at the end of the experiment. The washed cells are digested, converted to N_2 and analyzed for N^{15} content with a mass spectrometer.³

Ammonium- and Urea-N: Experiments 1 and 2, table 1, demonstrate how readily the NH_4^+ ion is utilized by *A. vinelandii*. In experiment 1 the organism had been previously grown on a nitrogen-free medium.

TABLE 1
COMPETITION OF AMMONIUM- AND UREA-N WITH N_2 AS SOURCE OF NITROGEN FOR
Azotobacter

EXPERIMENT	CULTURE "ADAPTED"* TO	NITROGEN SOURCE IN P. P. M. N	HOURS OF INCUBATION	ATOM % N^{15} EXCESS	PER CENT FIXATION
1	N_2	N_2		2.580.	100.0
		NH_4^+ —121	40	0.022	0.9
		NH_4^+ —315		-0.019	0.0
2	NH_4 acetate— 300 p. p. m.	N_2		26.600	100.0
		NH_4^+ —102	30	0.369	1.4
		NH_4^+ —312		-0.007	0.0
3	N_2	N_2		25.840	100.0
		Urea—97	47	2.530	9.7
		Urea—302		0.030	0.01
4	Urea—300 p. p. m.	N_2		27.140	100.0
		Urea—98	33	0.562	2.1
		Urea—296		0.101	0.4

* Immediately preceding their use, "adapted" cultures were carried through 3 daily transfers in media containing fixed N supplied by the N compound to be tested.

In experiment 2 the inoculum had been "adapted" by growth in 300 p. p. m. ammonium acetate nitrogen through 3 daily transfers. With either the "adapted" or "non-adapted" culture fixation was completely eliminated by 300 p. p. m. of NH_4^+ -N, and fixation accounted for only about 1 per cent of the cellular nitrogen in the presence of 100 p. p. m. NH_4^+ -N indicating that, when available, *A. vinelandii* uses the ammonium ion to the exclusion of N_2 .

The response of *A. vinelandii* to urea-N is very similar to that with NH_4^+ -N as can be seen by the data of experiments 3 and 4 in table 1. Even without previous culture on urea, the organism uses this source of nitrogen in preference to molecular N_2 ; after 3 transfers fixation is practically eliminated. The presence of an active urease in *Azotobacter* strongly suggests that urea serves essentially as another source of the NH_4^+ ion; the decrease in fixation after adaptation to urea may well arise from increased urease activity.

Nitrate- and Nitrite-N: The non-adapted culture (table 2, experiment 5) assimilated about 20 per cent of its nitrogen as N_2 in the presence of nitrate. However, after adapting the culture to nitrate, fixation was almost eliminated in the presence of NO_3 (table 2, experiment 6).

With a non-adapted culture (table 2, experiment 7) nitrite depressed fixation somewhat more than nitrate, but the culture adapted to nitrite responded in almost the same manner as the non-adapted culture (table 2, experiment 8). Nitrite levels above 75 p. p. m. N are toxic to *A. vinelandii* and hence nitrite and nitrate cannot be compared at the higher concentrations.

TABLE 2
COMPETITION OF NITRATE- AND NITRITE-N WITH N_2 AS SOURCE OF NITROGEN FOR
Azotobacter

EXPERIMENT	CULTURE "ADAPTED" TO	NITROGEN SOURCE IN P. P. M. N	HOURS OF INCUBATION	ATOM % N^{15} EXCESS	PER CENT FIXATION
5	N_2	N_2		2.110	100.0
		$NaNO_3$ —102	39	0.464	22.0
		$NaNO_3$ —277		0.381	18.1
6	$NaNO_3$ —300 p. p. m.	N_2		23.370	100.0
		$NaNO_3$ —100	33	0.476	2.0
		$NaNO_3$ —323		0.046	0.2
7	N_2	N_2		2.946	100.0
		$NaNO_2$ —50	15	0.420	14.2
8	$NaNO_2$ —50 p. p. m.	N_2		21.340	100.0
		$NaNO_2$ —66	29	2.600	12.2

Amino Acids: In the presence of molecular N_2 the dicarboxylic amino acids, aspartic and glutamic acids, are used to a very limited extent by a non-adapted culture, but when previously grown in the presence of these nitrogen compounds, *A. vinelandii* will use somewhat greater quantities of them (table 3, experiments 3 and 9). Likewise previous culture on asparagin (table 3, experiments 10 and 11) increases its utilization from a quarter to a half of the total nitrogen assimilated by *A. vinelandii*. As with urea we may logically consider the possibility that asparagin is functioning by furnishing the ammonium ion to the organisms, and that the adaptation consists in stimulating the formation of the enzyme splitting the amide linkage. The alternative, that the nitrogen used is amino, appears untenable because of poor utilization of aspartate. Arginine is utilized neither by adapted nor by non-adapted cultures (table 3, experiments 10 and 11). An acid hydrolyzate of casein was used to furnish a mixture of amino acids, but as is shown in experiment 12, *A. vinelandii* did not obtain an appreciable amount of its nitrogen from this source.

Discussion.—The primary objective of the studies with isotopic nitrogen is to provide better insight into the mechanism of biological nitrogen

fixation. In agreement with the previous investigations with this isotope, the data suggest, but do not establish, that ammonia may be a primary product of nitrogen fixation by *A. vinelandii*. Only ammonia and such compounds as are readily converted into ammonia by the organism compete to any marked extent with the N_2 fixation reaction. Evidently the bacteria possess the mechanism necessary to obtain *without delay* their entire nitrogen requirements from NH_4^+ . With NH_4^+ itself, and probably urea, the organism can change immediately from a metabolism involving solely N_2 to one in which NH_4^+ meets all needs. The rapidity of the change has not been determined exactly, but N^{15} can be detected in *A. vinelandii* cells within 1 minute after it is supplied as NH_4^+ to a N-free culture (unpublished data).

TABLE 3

COMPETITION OF AMINO ACIDS WITH N_2 AS SOURCE OF NITROGEN FOR *Azotobacter*

EXPERI- MENT	CULTURE "ADAPTED" TO	NITROGEN SOURCE IN P. P. M. N	HOURS OF INCUBATION	ATOM % N^{15} EXCESS	PER CENT FIXATION
3	N_2	N_2		25.84	100.0
		Aspartate—108	47	23.62	91.4
		Glutamate—102		25.01	96.7
9	N_2	N_2		26.73	100.0
	Aspartate—100 p. p. m.	Aspartate—102	41	22.52	84.2
	Glutamate—100 p. p. m.	Glutamate—137		23.80	89.0
10	N_2	N_2		3.329	100.0
		Arginine—50	24	3.486	104.8
		Asparagin—50		2.456	73.7
11	N_2	N_2		26.510	100.0
	Arginine—100 p. p. m.	Arginine—121	40	26.900	101.5
	Asparagine—100 p. p. m.	Asparagin—93		12.970	48.9
12	N_2	N_2		17.03	100.0
		Casein hydrol.—101	29	17.24	101.1
	Casein hydrol.— 100 p. p. m.	Casein hydrol.—101		16.52	97.0

(Sources of nitrogen which are more or less readily converted to NH_4^+ also definitely inhibit the fixation reaction.) Nevertheless, a significant difference in the inhibition is observed; if the culture has been previously restricted to N_2 , the organism does not immediately accept the combined nitrogen source to the complete exclusion of molecular nitrogen. If, however, a period of "adaptation" is provided (during which time the organism probably develops enough enzyme to convert the combined nitrogen source into ammonia at a rate sufficient to meet its needs), the

compound behaves as does ammonia, viz., it suppresses almost completely the fixation reaction.

The chief alternative to the hypothesis that biological nitrogen fixation proceeds via ammonia is the proposal of Virtanen⁴ that hydroxylamine is the key compound. His evidence is based almost entirely on the excretion phenomenon which occurs at times in leguminous plants. The arguments for and against this mechanism have been discussed by Wilson⁵ who emphasized that much of the evidence was non-specific in the sense that it could serve equally well if NH_4^+ were the prime intermediate. For example, independent of whether nitrogen is fixed as NH_4^+ or NH_2OH it probably is rapidly converted to amino acids, especially aspartic and glutamic acids, via the appropriate keto acid according to the generally accepted theory of nitrogen metabolism in plants. But it should be likewise noted that the data based on the isotopic research are also not critical in this same sense, viz., that they do not eliminate NH_2OH as a possible intermediate—only that nothing uncovered so far by this approach suggests the necessity of postulating its presence. Since even in dilute solution NH_2OH is toxic to *Azotobacter*, it has not been possible to test its effect on fixation in a manner comparable to that employed for other nitrogen compounds.

One noteworthy finding of the isotopic research should not be overlooked: neither aspartic acid, a key compound for the NH_2OH view, nor glutamic acid, a compound suggesting NH_4^+ , are utilized in the presence of N_2 . In both instances limitation in the cell's permeability may be concerned. Although both of these compounds undoubtedly must be used when synthesized by the cell itself, the organism does not normally grow in their presence probably because it lacks the necessary enzymes to secure immediately from them the key compound of its nitrogen metabolism—ammonia.

Summary.—*Azotobacter vinelandii* was grown in the presence of normal nitrogen compounds in an atmosphere containing N^{15} enriched molecular nitrogen. Isotopic analysis furnished a means for determining the ability of various compounds to compete with the nitrogen fixation reaction.

Ammonia and compounds readily converted to ammonia are used to the virtual exclusion of molecular nitrogen. With ammonium compounds, and probably urea, the change from a metabolism involving only N_2 to one based on combined nitrogen is rapid and complete. With other compounds, notably nitrate, a period of "adaptation" is essential, otherwise fixation is not entirely suppressed. With asparagin an increased but not complete inhibition of nitrogen fixation was observed after adaptation. Nitrogen compounds which the organism assimilates only with difficulty (aspartic and glutamic acids) or not at all (arginine) do not inhibit the fixation of N_2 to a marked extent.

The significance of these findings for the mechanism of nitrogen fixation by *Azotobacter* is discussed, and it is concluded that present evidence based on research with isotopic nitrogen favors the view that NH_4^+ is a key intermediate.

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COLCHICINE-INDUCED ALLO- AND AUTOPOLYPLOIDY IN *NICOTIANA**

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For many years there has been intensive investigation of the genus *Nicotiana* designed to throw light upon origins and relationships of the 60 to 70 species as well as having to do with the detection or improvement of species of economic importance. It is not surprising, then, that the announcement of the successful use of colchicine in doubling chromosome number should have prompted colchicine treatment of *Nicotiana* species and hybrids, to various ends.

First, the possibility of artificially inducing chromosome doubling is important as bearing upon the hypothesis that the 24-paired species of *Nicotiana* are of amphidiploid origin involving progenitors of 12-paired species (cf. Goodspeed,^{1,2} Clausen³). Thus, the two species of commerce, *N. Tabacum* and *N. rustica*, are looked upon as having been derived from hybridization followed by chromosome doubling. In the origin of *N. rustica* members of the "paniculata group"† were involved—probably the progenitors of the modern species *N. undulata* and *N. paniculata* (Goodspeed,¹ Kostoff,^{4,6} Goodspeed and Bradley⁷). Species of the "tomentosa group" and *N. sylvestris* have undoubtedly entered into the amphidiploid origin of *N. Tabacum* (Goodspeed and Clausen,⁸ Kostoff,⁹ Clausen,¹⁰ Goodspeed,¹ Greenleaf,¹¹ Goodspeed and Bradley⁷). In the origin of 24-paired *N. Bigelovii*, *N. nudicaulis*, *N. repanda* and *N. nesophila* species of the 12-paired "acuminata" and "alata groups," certain of which may no longer exist, are thought to be involved. A new 24-paired species, *N. Arentsii* (Goodspeed²) has been shown to be a product of hybridization between progenitors of *N. undulata* and *N. wigandioides* followed by chromosome doubling. This discovery of a native species of *Nicotiana* of demonstrated amphidiploid origin has importance in connection with the accumulated cytogenetic evidence indicating such origin in the case of *N. Tabacum* and *N. rustica*.

In the second place, colchicine-induced chromosome doubling of species and hybrids of *Nicotiana* holds out possibilities of economic importance. Thus, new races possessing desirable qualities of aroma or combustibility, altered contents of nicotine, nicotinic acid and related substances (cf. Smith and Smith,¹² Noguti, Oka and Otuka¹³) or increased disease resistance (cf. Holmes¹⁴) might be artificially produced via allo- or autopolyploidy.

Among the previously reported *Nicotiana* amphidiploids obtained by colchicine treatment of F_1 hybrids are: *Nicotiana excelsior* \times *N. velutina* (Kostoff¹⁵), *N. glutinosa* \times *N. glauca* (Smith¹⁶), *N. glutinosa* \times *N. sylvestris* (Warmke and Blakeslee¹⁷), *N. glutinosa* \times *N. Tabacum* (Mendes¹⁸), *N. rustica* \times *N. Tabacum* (Smith¹⁶), *N. suaveolens* \times *N. alata* (Kostoff,^{6,15}), *N. suaveolens* \times *N. Sanderae* (Kostoff⁶), *N. Tabacum* \times *N. glauca* (Smith¹⁶), *N. Tabacum* \times *N. glutinosa* (Warmke and Blakeslee¹⁷), *N. Tabacum* \times *N. sylvestris* (Bartolucci,¹⁹ Smith,¹⁶) and *N. alata* \times *N. Sanderae* (Kostoff¹⁵). Colchicine-induced autotetraploids include: *N. Sanderae* (Warmke and Blakeslee,¹⁷ Kostoff¹⁵), *N. glauca* (Smith,¹⁶ Kostoff¹⁵). A number of these same F_1 hybrids and species have reacted similarly to colchicine in the work with this treatment that has been under way in the University of California Botanical Garden for a number of years and in which the following additional allo- or autotetraploids have been obtained: *N. maritima* \times *N. plumbaginifolia*, *N. otophora* \times *N. Setchellii*, *N. paniculata* \times *N. solanifolia*, *N. rustica* \times *N. paniculata*, *N. Tabacum* \times *N. otophora*, *N. Benthamiana* \times *N. Debneyi*, *N. maritima* \times *N. velutina*, *N. velutina* \times *N. suaveolens*, *N. velutina* \times *N. rotundifolia*, *N. alata* and *N. paniculata*. Viable seed has been obtained from all. Two additional polyploids, $4n$ *N. Setchellii* and *N. "ditagla"* (an amphidiploid of F_1 *N. Tabacum* \times *N. glauca*) \times *N. sylvestris* were induced, but the plants died before seed was procured.

Types of Colchicine Treatment.—Initially, drops of 0.4% colchicine solution were applied to the vegetative buds of a total of 17 plants of F_1 interspecific hybrids of *Nicotiana*. A number of buds on each plant were treated twice every other day for from eight to ten days. Of the relatively few buds which survived the treatment, practically all gave rise to branches which showed the mixochimerical effects characteristic of tissues derived from colchicine-treated meristems.

Five of the 17 plants produced branches which bore flowers with doubled chromosome numbers. In this and in all other cases pollen size and condition were used as preliminary indication that such doubling had occurred. Chromosome counts and fertility confirmed such preliminary evidence. The successfully treated F_1 hybrids were *N. Benthamiana* \times *N. Debneyi*, *N. maritima* \times *N. velutina*, *N. velutina* \times *N. suaveolens*, *N. velutina* \times *N. rotundifolia* and *N. paniculata* \times *N. solanifolia*.

Thirty-nine plants, most of them F_1 hybrids, were treated by the capillary string method with a 0.3 to 0.5% colchicine solution, the treatment lasting from 2 to 5 days. Many buds were killed, particularly those subjected to a 0.5% solution for 5 days. Branches with doubled chromosome numbers appeared on two plants. One of these, F_1 *N. Tabacum* \times *N. otophora*, developed two tetraploid branches, following 0.4% colchicine for 4 days and 0.5% for 3 days, respectively. On another, F_1 *N. rustica* \times *N. paniculata*, a tetraploid branch arose from a bud treated with 0.5 colchicine for 3 days.

An emulsion containing 0.4% colchicine (cf. Warmke and Blakeslee¹⁷) was applied to vegetative buds of fifty-five plants representing twenty-one populations of species and F_1 hybrids. Applications of the emulsion were made twice a week until the effects of colchicine became evident in the checking of growth and thickening of young leaves. Four plants—two of *N. Sanderæ* and two of *N. alata*—have so far produced tetraploid branches.

For the above series of colchicine treatments the results are not entirely reliable since treatment was in many instances applied when the plants were not in an actively growing condition or when environmental factors were more or less unfavorable for growth. The importance of optimum conditions for growth and of employing plants in early stages of maturity for success in inducing polyploidy is now well recognized.

Two colchicine techniques were entirely unsuccessful—seed treatment and injections into stems near buds. Seeds of one species and of three F_1 hybrids were placed on filter paper saturated with 0.5% colchicine. Two lots were treated for 48 hours and two for 72 hours before the seeds were sown on soil. No germination occurred, although controls showed a high percentage germination. Injections just below and above buds of 21 plants failed to induce chromosome doubling. Solutions of 0.4% colchicine, in some cases made up with 0.2% alkanol, were injected. Some buds failed to produce branches, probably as a result of mechanical injury.

Maturing seed capsules of 11 species and F_1 hybrids were injected (cf. Nebel and Ruttle²⁰) with a solution of one part 0.8% colchicine and one part 0.2% alkanol. The size of the capsules injected varied from those approximately half that attained at maturity to mature but still green ones. The treatment completely inhibited seed development in the youngest capsules and, in most cases, injected seed capsules either abscised before maturity or contained only aborted seed. In two instances a little viable seed was obtained but the plants grown from it died in the seedling stage. A number of injected capsules on single plants of F_1 *N. Benavidesii* \times *N. solanifolia*, F_1 *N. otophora* \times *N. Setchellii* and of *N. otophora* produced viable seed from which small populations were grown. In all three there were plants which grew and flowered normally and proved to be diploid

and others which developed so slowly that they did not flower before the end of the growing season. These results indicate the possible importance of further examination of the capsule injection technique

Treatment of seedlings gave more consistent and significant results than any other colchicine technique employed. Seeds were sown on moist filter paper. When germination had proceeded to the point that seed coats were being shed (an over-all length of 0.5–1.0 cm.), the seed was transferred to filter paper saturated with 0.5% colchicine solution. They remained in contact with this solution for periods of two, three, four and five days. Of a total of approximately 250 seedlings of seven F_1 hybrids and ten species that were treated, 23% survived to maturity and of this number 5% showed complete or partial chromosome doubling. Five-day treatment of a total of 52 seedlings of three F_1 hybrids and one species gave only 4 mature plants, all of F_1 *N. rustica* \times *N. paniculata*. One of these plants contained a $4n$ sector. Three- and four-day treatment proved to be most effective and in every case where mature plants were produced one or more of them showed complete chromosome doubling or $4n$ sectors. Following two-day treatment two species, *N. Setchellii* and *N. paniculata*, gave mature plants showing induced polyploidy. In the case of the species, the seedlings of which were treated, a considerable difference in susceptibility to the injurious effects of colchicine was indicated by the distinctions in survival following a given treatment.

For bud treatment it was clear that, as already noted, plants in anything but a condition of active growth are unfavorable subjects. Prolonged treatment and low concentrations of colchicine are necessary in the case of semi-dormant plants. Since, however, the toxic effects of colchicine are doubtless slowly dissipated when growth is slow, such dosages are difficult to regulate and may become too drastic with consequent death of treated meristems. In *Nicotiana*, treatment of a few lateral buds and elimination of all others does not appear to encourage the growth of treated buds since adventitious buds near the eliminated ones are at once stimulated to growth. Severe topping followed by treatment of all remaining buds has proved to be the most favorable technique. The number and hairiness of foliage leaf primordia obviously affect rate and degree of penetration into bud meristems of colchicine solution applied by the drop or capillary string methods. These methods were largely unsuccessful except with species and F_1 hybrids of the "suaveolens group" and of certain members of the "paniculata group" where, probably, the number of leaf primordia and certainly the density of the indument is reduced as contrasted with most of the species of other genetic groups that were treated.

Dosages in seedling treatment of *Nicotiana* species should be based upon the differential susceptibility of various species to the toxic effects of colchicine. Such differences are, apparently, both inherent and also related

to the readiness with which untreated seedlings can be reared to maturity. Colchicine concentrations of less than 0.5% in contact with seedlings for longer periods than those employed by us may give a higher percentage of survival and greater incidence of polyploidy than reported above. However, heavy dosage followed by a low survival rate and a fair proportion of successful treatments reduces time and expense in carrying on such investigations.

Complete cytogenetic analysis of C_1 and later generations of colchicine induced auto- and allotetraploidy will be made only when such evidence contributes to other types of investigations in progress. Certain information is, however, in hand. Thus, the induced autotetraploids of species show the anticipated coarsening of general habit, increase in size and thickness of leaves and flowers and decreased rates of development. A $4n$ plant of *N. glauca* exhibited those tetraploid characteristics but was otherwise normal in external morphology and growth reaction. Like those of the other induced autotetraploids, its PMC meiosis exhibited a certain degree of multivalent formation. However, in the next generation (C_1) developmental abnormalities uniformly occurred. On young plants the leaves were extremely thick and leathery while the terminal shoot and laterals showed a tendency toward fasciation and bore slender to ribbon-like, distorted leaves. Gradually these abnormalities were outgrown but up to the end of the season no flowers were produced. Genic effects noted in F_1 interspecific hybrids involving certain races of *N. glauca* and resulting in developmental abnormalities were probably operative in this instance also.

In the case of F_1 hybrids in which chromosome doubling was induced the cytogenetics of the original amphidiploid determined the C_1 and C_2 conditions observed. For example, *N. paniculata* ($n = 12$) and *N. solanifolia* ($n = 12$) have fundamentally the same genic constitution as indicated by an average of 11.8 pairs per PMC in the F_1 hybrid between them. This hybrid in the doubled condition shows from 2 to 7 multivalent configurations and one or more univalents per PMC. In a population of 30 C_1 plants habit and leaf characters were practically uniform in expression but rate of growth and a number of floral characters showed considerable segregation. In C_2 segregation corresponding in nature to that of C_1 occurred. The segregating characters were those on the basis of which the parental species are distinguished taxonomically and the substitution or elimination, following multivalent and univalent distribution, of chromosomes bearing gene complexes of which these characters are a reflection would produce the variability observed in C_1 and C_2 .

By contrast, *N. maritima* ($n = 16$) and *N. plumbaginifolia* ($n = 10$) are widely separated in genic constitution. In the amphidiploid of their F_1 hybrid only bivalents appeared in PMC and correspondingly the C_1 population was uniform in morphological characters.

Finally, there were such F_1 hybrids as *N. rustica* ($n = 24$) \times *N. paniculata* ($n = 12$) n and *N. Tabacum* ($n = 24$) \times *N. otophora* ($n = 12$) in which Drosera scheme chromosome behavior occurs. The original amphidiploid of the former hybrid exhibited multivalent formation as a result of allo- and autosyndesis and irregularities in chromosome distribution followed by formation of micronuclei and microcytes at the quartet stage. Although, doubtless, many products of such meioses are inviable, certain gametes with slightly deficient chromosome numbers or with extra chromosomes should be viable. That this is the case was indicated by the considerable amount of segregation in many plant characters in a C_1 population of 40 plants. Probably this colchicine induced amphidiploid is comparable to that of Lammerts^{21,22} in which he found that dissociation of quadrivalents was not strictly preferential and that complete substitution is possible, in some quadrivalents at least. Since Lammerts produced some stable 72 chromosome derivatives, selfing of the colchicine induced *rustica-paniculata* amphidiploid and its progenies should likewise yield stable lines. A C_1 population of 25 plants of F_1 *N. Tabacum* \times *N. otophora* showed a variability comparable to that of the hybrid just referred to, along with a comparable meiotic condition in the original amphidiploid plant.

The results above listed emphasize the comparative readiness with which auto- and allopolyploidy may be induced in *Nicotiana*. Reference has above been made to the application of natural and induced polyploidy in tobacco breeding and in the interpretation of species origins and relationships. In this latter connection the cytogenetic significance of polyploidy in the genus *Nicotiana* has also been discussed by Clausen.³

We are indebted to P. C. Burrell for assistance in the colchicine investigations.

(Since the above was written vegetative buds of 16 plants of F_1 *N. sylvestris* \times *N. otophora* have been treated with a 0.4% solution of sanguinarine sulfate. Little,²³ using sanguinarine hydrochloride, produced tetraploidy in *Antirrhinum majus*. Although amphidiploidy in F_1 *N. sylvestris* \times *N. otophora* has not as yet been induced, shoots from the injected buds show abnormalities in development equivalent to those following colchicine treatment. Probably, therefore, either salt of sanguinarine properly applied will induce chromosome doubling.)

* Contribution No. 111 from the University of California Botanical Garden.

† The nature and significance of the genetic group concept are referred to elsewhere (Goodspeed^{1,4}).

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CHROMOSOMAL ABERRATIONS IN BRAZILIAN *DROSOPHILA ANANASSAE*

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Drosophila ananassae de Meijere is a species common in or close to human habitations in the tropical and subtropical parts of the world. Most of its relatives are confined to eastern and southern Asia. It seems reasonable to suppose that *D. ananassae* is also a native of these lands; its presence elsewhere, particularly in the Americas, is due to introduction by man. A study of the chromosomal variability confirms this conjecture. As shown below, Brazilian populations contain mostly the same chromosome variants which occur in southeastern United States and in eastern Asia. Such a uniformity of intraspecific chromosome variants could hardly occur if the present wide distribution of the species would be entirely a "natural" one. In one of the Brazilian populations we have, however, established the presence of translocation heterozygotes. While inversions occur in most species of *Drosophila*, this is the first instance of a translocation being found in a population of *Drosophila* outside genetic laboratories.

We have received population samples of *D. ananassae* from Bello Horizonte (state of Minas Gerais) collected by Mr. José Pellegrino, from Rio de Janeiro collected by Professor Hugo Souza Lopes, from Mogi das Cruzes

collected by Mr. C. Pavan, from Santos and São Vicente collected by Mr. Aylthon Joly, and from Itanhaem collected by Mr. Edmundo Nonato; the last four localities are in the state of São Paulo. *D. ananassae* has four pairs of V-shaped chromosomes at metaphase, represented in the salivary gland chromosomes by four long (autosomal), two shorter (*X*-chromosome), and one very short (autosomal) chromosome strands. A study of the salivary gland chromosomes of the offspring of females collected outdoors showed that only about 7 per cent of the individuals (8 out of 118) are free of inversions, while the remainder are heterozygous for from one to four inversions. The commonest inversion is the subterminal one in the left

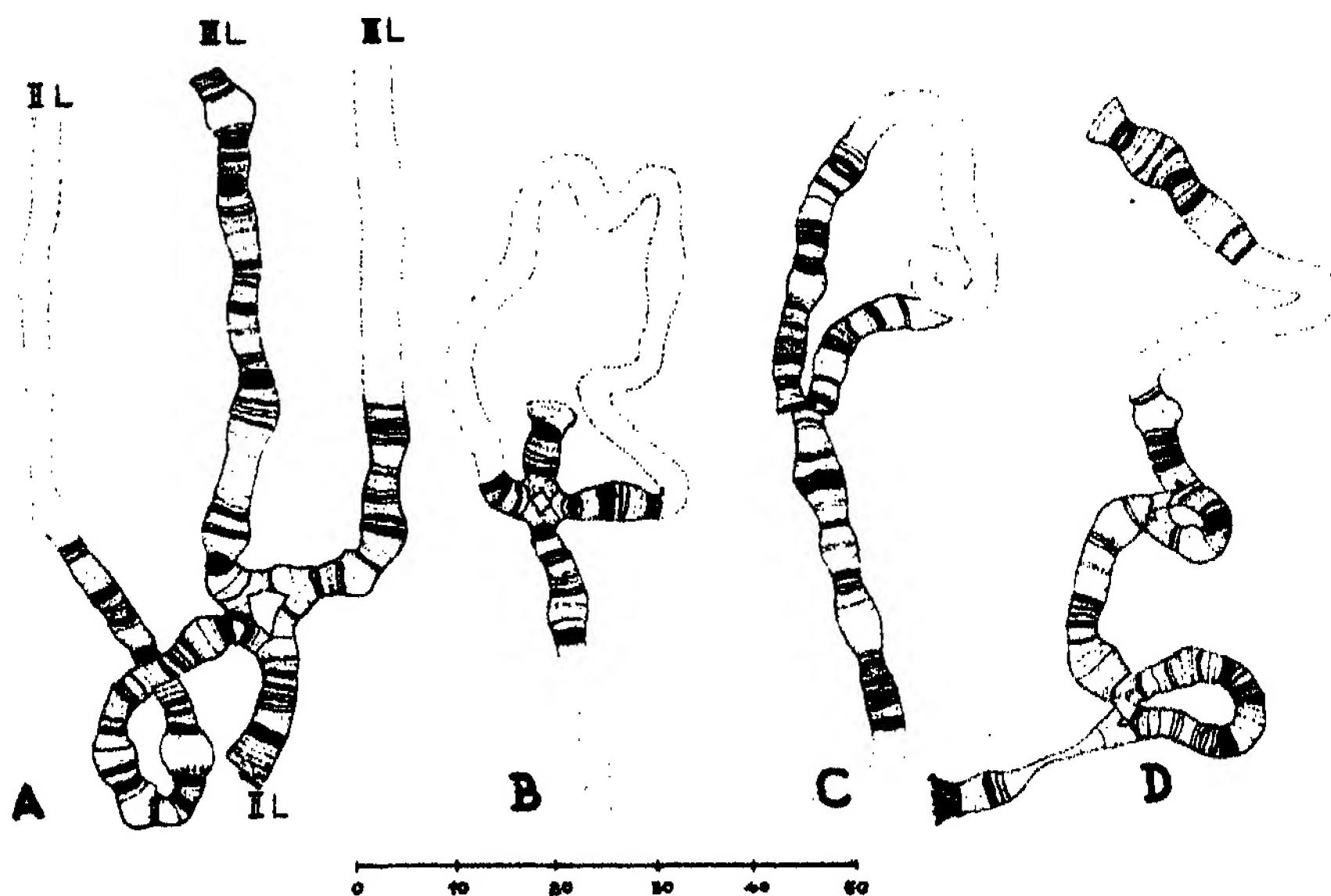


FIGURE 1

A, a translocation between the left limbs of the second and the third chromosomes. Note a deficiency of the terminal disc on one side in the tip of II L. B, subterminal inversion in II L. C, apparently terminal inversion in III L. D, basal and median inversions in III R. The scale represents 50 micra.

limb of the second chromosome (II L) shown in figure 1, B; about one-half of individuals in all the localities are heterozygotes for the inversion. The next commonest is the apparently terminal inversion in the left limb of the third chromosome (III L) shown in figure 1, C; about 40 per cent of the flies in all the localities are heterozygotes for this inversion. The small inversion in the basal part of the right limb of the third chromosome (III R basal, Fig. 1, D) was also found in all localities, but only about 30 per cent of the flies are heterozygotes. A small inversion in the middle part of the right limb of the third chromosome (III R median, Fig. 1, D) occurred in

three individuals from Bello Horizonte. Finally, an inversion in the middle portion of the right limb of the second chromosome (II *R*, figured by Kikkawa¹ on his plate I *J*) occurred in several individuals from Mogi das Cruzes, Rio de Janeiro, Santos and São Vicente. Kaufmann² found in Alabama populations the inversions II *L*, III *L*, II *R* and III *R* basal. Kikkawa¹ found in populations of several localities in Japan and in Shanghai, China, the inversions recorded by Kaufmann in Alabama, plus a small

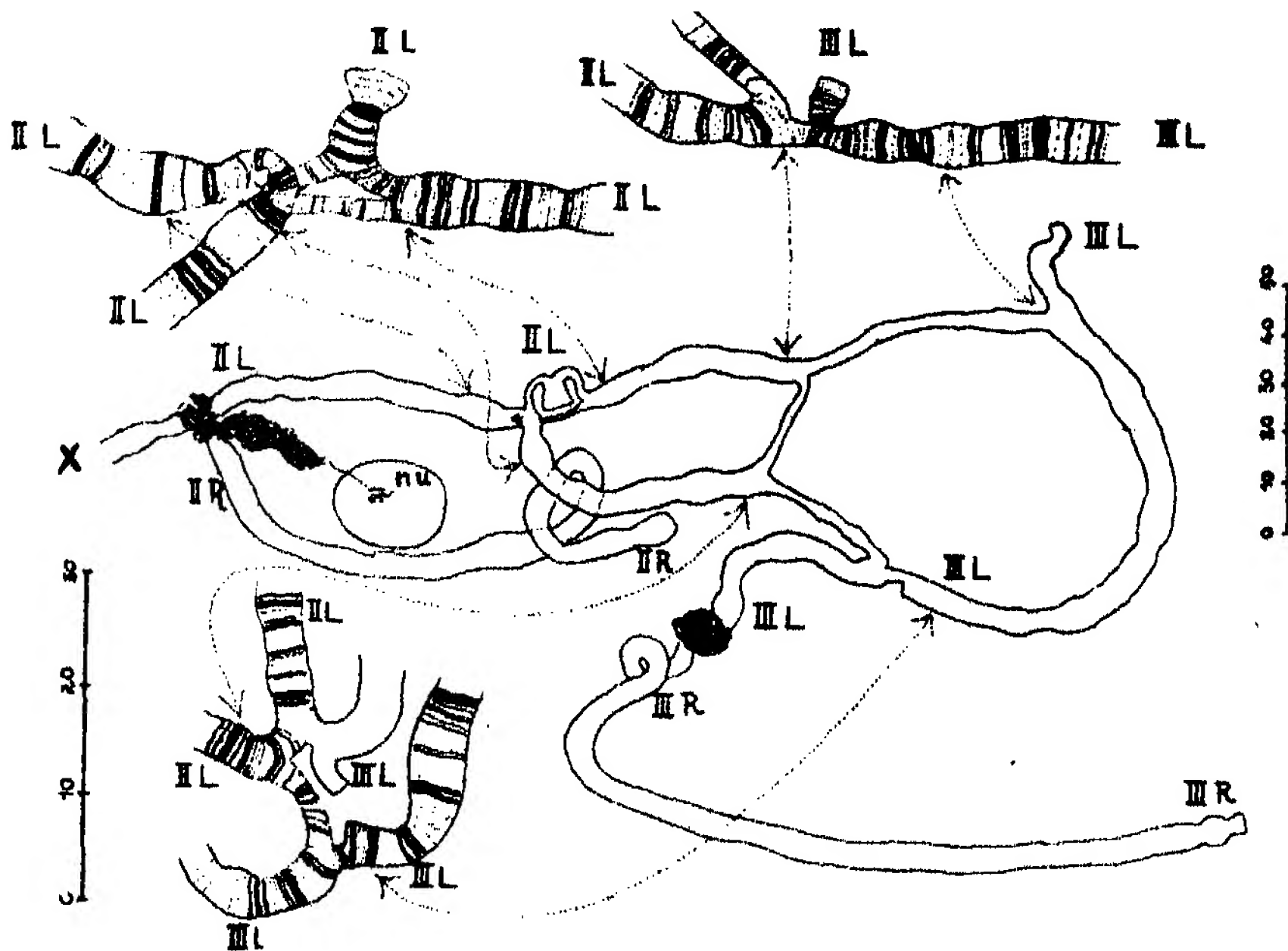


FIGURE 2

In the center, an outline drawing of the configuration resulting from a combination of the translocation and two inversions in the left limbs of the second (II *L*) and the third (III *L*) chromosomes; II *R* and III *R*, respectively, the right limbs of the second and the third chromosomes; X, X-chromosome; nu, nucleolus; the chromocenter is shown as a body with a reticulate structure; the magnification is indicated by the scale on the right representing 50 micra. The drawings above and below the outline show details of the structure of the critical portions as seen in other cells; the scale on the left represents 30 micra and is applicable to the detailed drawings.

inversion in II *L* in a single strain from Taihoku recorded neither by Kaufmann nor by ourselves. Our III *R* median inversion from Bello Horizonte is, thus, the only not previously known one. The translocation between the third and the fourth chromosomes described by Kikkawa¹ is a laboratory product.

In a strain coming from one of the seven females collected at Mogi das Cruzes, there occurred the highly complex chromosome configuration rep-

resented in outline in figure 2. The left limbs of the second and third chromosomes are, in this configuration, taking part in the formation of two closed rings; the right limbs of the second and the third, and the *X* and fourth chromosomes are normal. A careful examination of the disc patterns in the critical regions of the chromosomes in the configuration just referred to (see the drawings in Fig. 2 showing the disc patterns; these drawings have been made from several cells, at a magnification higher than the outline in the central part of this figure) has led to the conclusion that a reciprocal translocation between the left limbs of the second and third chromosomes as well as the II *L* and III *L* inversions are involved. An interpretation of the configuration of figure 2 is shown schematically in figure 3. In this latter figure the normal left limb of the second chromosome is shown dotted; the normal left limb of the third chromosome black; the left limbs of the second and third chromosomes involved in the translocation white and cross-hatched, respectively. The base and a part of the inverted portion of the second chromosome (white) has acquired most of the inverted terminal portion of the third chromosome (cross-hatched); the base of the third chromosome (cross-hatched) has acquired part of the inverted portion and the uninverted tip (white) of the second chromosome.

Chromosomes with and without the II *L* and III *L* inversions are both found in the populations studied by us. Therefore, if the interpretation given to the configuration shown in figure 2 is correct, we should be able to find much simpler configurations showing the translocation with only II *L*, or only III *L* inversion, or without either inversion. The characteristics of these configurations are predictable, and they have indeed been found in the salivary glands of other larvae from the same culture which showed the configurations of figures 2 and 3, as well as in another culture obtained from a female caught at Mogi das Cruzes. Figure 1, *A* shows the translocation configuration without the complicating inversions. This is a typical cross-shaped configuration observed in translocation heterozygotes.

It appears, then, that two of the seven females collected at Mogi das Cruzes were either translocation heterozygotes or have mated with translocation males. Unfortunately, the translocation stock has been lost before the viability of the translocation homozygotes and related problems could be studied; new population samples from the same locality have as yet not been obtained. Since translocation heterozygotes in *Drosophila* usually have a fertility below that of homozygotes, translocations are not expected to be retained in natural populations, and the case just described is so far the only one on record.

Other "forbidden" classes of chromosome changes are terminal deficiencies, duplications and inversions. The terminal chromomere ("telomere") is supposed to be a structural element without which a chromosome cannot exist, and which cannot be replaced by a chromomere usually oc-

cupying an interstitial position. The III *L* inversion has been described by Kaufmann² as a terminal one, and we can confirm that it is visibly so. Kikkawa¹ described variations in the structure of the free ends of the second and third chromosomes in *D. ananassae* which appear as deficiencies of a single or a few terminal discs (or as additions of new discs not present in that location in other strains). We find in Brazilian populations variations apparently identical with those described by Kikkawa. An instance of a heterozygous deficiency for a terminal disc in the left limb of the second chromosome is shown in figure 1, *A*. The nature of these terminal deficiencies (or duplications) is unclear, but in any case they have nothing to do with the "pseudotranslocations" allegedly found by Goldschmidt³ in *D. melanogaster*. Analogous variations have been found by Metz⁴ in species of *Sciara*. If the free ends of chromosomes are heterochromatic, we may be

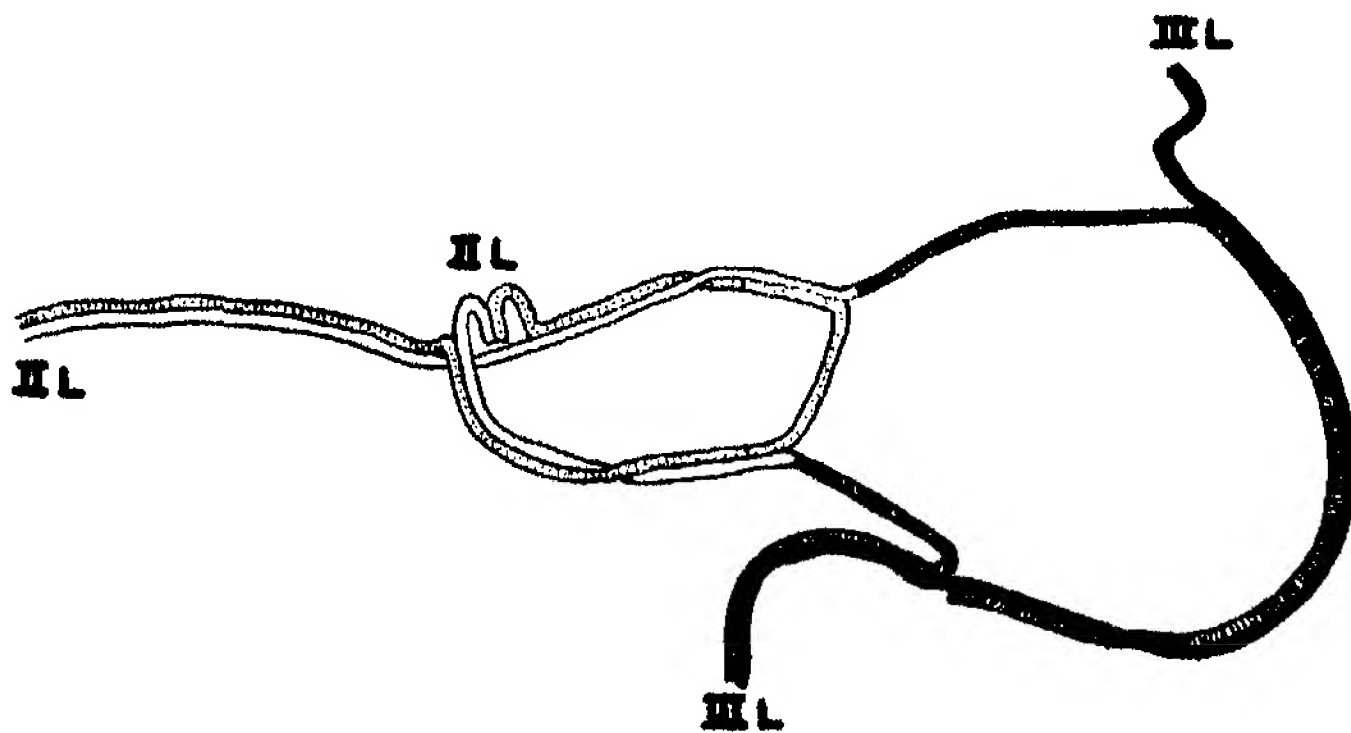


FIGURE 3

A scheme of the configuration shown in figure 2. The left limbs of the second and third chromosomes not involved in the translocations are shown white and black, respectively; those suffering translocation, dotted and cross-hatched, respectively.

dealing with real deficiencies of elements that are no longer essential constituents of the germ plasm. Or else, these may be cytological visible results of changes of the type of gene mutations. Such changes are, as well known, not usually reflected in the appearance of the chromosomes, but cytologically visible effects of gene mutations are theoretically not excluded. In any case, these variations seem to be terminal, although, of course, we cannot exclude the existence of an invisible telomere persisting at the end of the chromosome despite the visible variations.

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A NEW METHOD FOR HYBRIDIZING YEAST

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Winge and Laustsen¹ hybridized yeasts by placing a haploid ascospore from one strain in close proximity to an ascospore of a second strain by use of the micromanipulator. When all conditions are favorable, the two spores fuse to produce a diploid hybrid cell. We² have used this method extensively, and have found that copulation usually fails (1) if the two spores are of the same mating type, (2) if either of the spores germinates directly into a diploid cell (this happens rather frequently, especially in vigorous strains), (3) if either spore is inviable (viability is generally about 50 to 75 per cent). These facts mean that relatively few hybrids are obtained by ascospore to ascospore matings. The method has the further disadvantage that the characteristics of the parents cannot be determined, since the single haploid spore used as a parent is consumed in the mating process. This is particularly important in the yeasts which are extremely heterozygous.

We have developed a new procedure based on the fact that some single ascospores from *Saccharomyces cerevisiae* produce persistently haploid cultures. It is possible to hybridize these with other similarly derived persistently haploid cultures simply by mixing the cells together in an appropriate medium. These mixtures result in copulation if each culture is paired with a complementary type. One test tube can be used for large number of matings. This experiment has given further support to our proposed scheme of alleles controlling copulation in *S. cerevisiae* and other indications prove that a simple allelism obtains. Morphology and biochemical characteristics can be studied previous to matings, and matings made between complementary types result in copulation tubes and diploid zygotes.

The four spores from one single ascus all produced persistently haplophase cultures. The four single spores were designated, *A*, *B*, *C* and *D*, and the haplophase cultures were paired in all combinations. *A* and *D* were found to belong to the same mating type, while *B* and *C* belonged

DESCRIPTION OF FIGURE 1

The four stable haplophase single ascospore cultures derived from a single ascus of *Saccharomyces cerevisiae* (*A*, *B*, *C* and *D*) and the results of pairing these cultures in all possible combinations. *A* × *D* and *B* × *C* pairings do not result in copulations. The *A* × *B* pairing results in copulation, and the zygotes are much larger than the cells found in either *A* or *B* alone, suggesting that the hybrid is more vigorous than either parent. The same increase in size is observed in the *C* × *D*, *A* × *C* and *B* × *D* pairings.

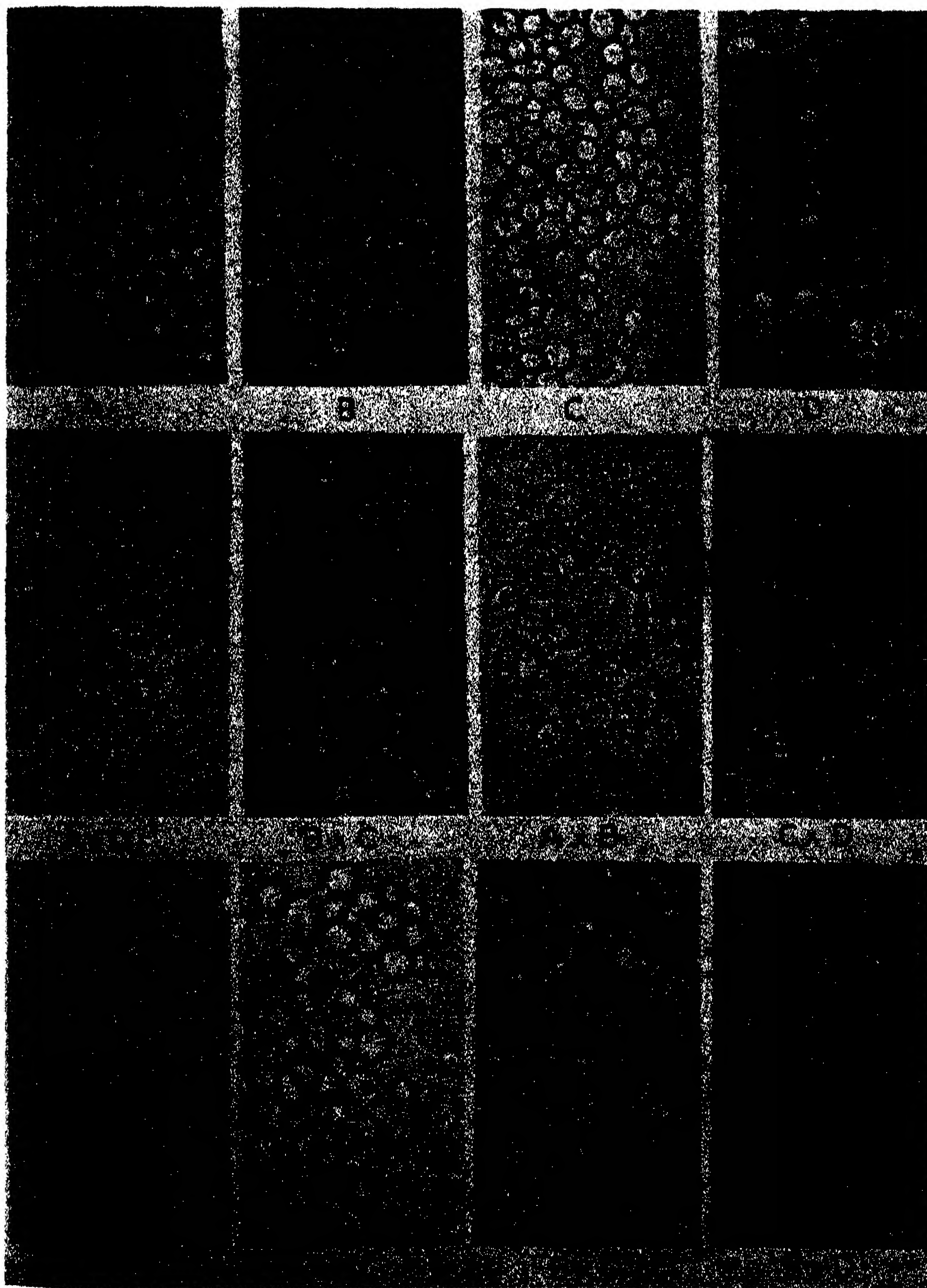


FIGURE 1 (Description on Opposite Page)

to the complementary type. Copulation tubes and zygotes were produced when A and B , A and C , B and D , and C and D were paired. These results are shown in figure 1. When transferred to gypsum,⁸ the diploid cells produced 4-spored asci, while the A and D , B and C combinations failed. No spores were obtained from any of the unmated single ascospore cultures.

We have already confirmed the views of Winge⁴ and Satava⁵ that the round-celled members of the genus *Torula* are imperfect forms derived from the genus *Saccharomyces*. In the present experiment these *Torulæ* when properly paired produced copulation tubes according to the pattern of the genus *Zygosaccharomyces*, indicating that it is also identical to *Saccharomyces*.

* This work was supported by a grant from Annheuser-Busch, Inc., St. Louis.

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SPECIAL INVARIANT SUBGROUPS

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The concept of invariant subgroup but not the name thereof was introduced by E. Galois, who considered the subgroups which have the property that when in a finite group G all the elements of the given group are arranged in the form of a rectangle with respect to the subgroup H as the first row, then all the co-sets, or rows, thus obtained are the same independently of whether these elements are arranged in the form of right co-sets or in the form of left co-sets with respect to H . If all these co-sets are the same in these two arrangements then H is now commonly called an invariant subgroup of G , but if not all of them are the same then H is called a non-invariant subgroup of G . E. Galois did not give a special name to these subgroups but he spoke of the decomposition of G with respect to such subgroups and it is on this account that the concept of invariant subgroup is now credited to him.

A considerable number of other terms have been used instead of the term invariant subgroup. Among these are the terms self-conjugate subgroup

and normal subgroup. The term invariant subgroup was used by the Norwegian mathematician S. Lie in 1878 and was later rapidly adopted by other writers on group theory on account of the great influence of the writings of S. Lie on the early development of group theory and its wide applications. Although the concept of invariant proper subgroup is very useful it should be noted that it does not apply to the important category of groups known as simple groups of composite order which are of fundamental importance in the theory of the solutions of algebraic equations and involve the greatest difficulties of group theory.

The most important special type of invariant subgroups is known as characteristic subgroups. In this case both the concept and the name are due to the same writer, viz., G. Frobenius, who introduced this term in 1895 and who used the concept extensively in his later writings on group theory. This subgroup is invariant in the holomorph of the group and corresponds to itself in every automorphism of the group. While the term invariant subgroup relates to the behavior of a subgroup with respect to the operators of the group itself the term characteristic subgroup relates to the behavior of a subgroup with respect to operators which are not in the group but which transform the group into itself. A necessary and sufficient condition that a subgroup is a characteristic subgroup is that it is transformed into itself by every operator which transforms the group into itself.

We proceed to consider the category of groups which separately satisfy the condition that they contain at least one invariant proper subgroup without also containing at least one characteristic proper subgroup. We shall first prove that such a group G cannot be non-abelian. It is well known that the non-abelian groups whose orders are divisible by two and only two prime numbers separately contain a characteristic subgroup whose order is one of these numbers. We may therefore assume that there is no non-abelian group whose order is divisible by a smaller number of prime numbers than the order of G but which has the property that it contains an invariant proper subgroup but no characteristic proper subgroup. If H is an invariant proper subgroup of G then the quotient group of G with respect to H must be an abelian group since it contains at least one invariant proper subgroup because G contains more than one invariant proper subgroup which is simply isomorphic with H because H is not a characteristic subgroup. The quotient group of G with respect to H must therefore be an abelian group of order p^m and of type 1^m , p being a prime number.

The order of the given G could not be a power of p since otherwise the central of G would be a characteristic proper subgroup of G . Since the quotient group of G with respect to H is of order p^m and G contains at least one invariant subgroup which is similar to H it results that the operators of H whose orders are prime to p generate a proper subgroup of H which is

the cross-cut of H and another invariant subgroup of G . This cross-cut could not be a characteristic subgroup of G since it was assumed that G does not contain such a subgroup. We have therefore arrived at contradiction by assuming that G is non-abelian, and when G is abelian and has the property that it contains at least one invariant proper subgroup but no characteristic proper subgroup it is obviously the abelian group of order p^m and of type 1^m . That is, *when a group contains an invariant proper subgroup but no characteristic proper subgroup it is the abelian group of order p^m and of type 1^m , p being a prime number.*

A useful illustration of the concept of characteristic subgroups is furnished by the dihedral groups. If the order of such a group is twice an odd number then every proper subgroup of the cyclic subgroup of index 2 of this dihedral group is a characteristic subgroup of the group and these subgroups are also the only invariant subgroups of the group. On the other hand, the dihedral group whose order is divisible by 4 and exceeds 4 contains also as characteristic proper subgroups all the proper subgroups of its cyclic subgroup of index 2. In this case there are, however, two additional invariant subgroups in the group which are not also characteristic subgroups of the group. These two subgroups are simply isomorphic because they are dihedral and of the same order, each having an order which is one-half the order of the group. They can be made to correspond in a simple isomorphism of the group with itself because their operators of order 2 which are not contained in the cyclic characteristic subgroup of index 2 transform the operators of this subgroup in the same way.

Three special types of characteristic subgroups which have received considerable attention are the commutator subgroups, the central subgroups and the norms, or character subgroups. The first of these correspond to the identity in the largest abelian quotient group, the second correspond to the group of inner isomorphisms of the group and are composed of all the operators of the group which transform into itself every operator of the group, while the third are composed of all the operators of the group which transform into itself every subgroup of the group. The norm clearly always includes the central of the group but it may also contain other operators of the group. In the case of the quaternion group, for instance, the norm is the entire group, while the central is the subgroup of order 2 contained therein. In the group of the square, or the octic group, the norm and the central are the same subgroup.

Just as the concept of invariant subgroup was introduced before the term invariant subgroup was used, so the concept of commutator subgroup was introduced before the term commutator was used. According to E. Vessiot the present writer gave effectively the present common name to this concept¹ but it should be noted that the term commutator was used earlier by R. Dedekind. The concept of the commutator of two elements of a group

had been used earlier by C. Jordan and A. Bochert in the theory of finite groups while S. Lie had made much use of it in the theory of continuous groups and suggested its usefulness in the theory of finite groups. The concept of commutator is much older than that of commutator subgroup. The main properties of the commutator subgroup were first published in the *Quarterly Journal of Mathematics*, 28, 266 (1896), by the present writer and have since then been widely used.

The history of the special invariant subgroups has been obscured by some statements in an article which appeared in volume 1 of the *Compositio Mathematica*, 1935. On page 254 of this volume it is asserted that essentially two types of invariant subgroups of an arbitrary group are known, viz., the commutator subgroups and the centrals. It should be noted that both of these two types of subgroups are special cases of the invariant subgroups known as characteristic subgroups but that a group may have more than one such invariant subgroup and that these invariant subgroups had received their now common name in the group theory literature before the commutator subgroups or the centrals had been thus named. The ϕ -subgroup should also have been noted here. The concept of norm is obviously very closely related to that of central and was introduced by R. Baer under the name of *Kern* in the article cited above. The term norm² was used for this concept by the same writer in the *American Journal of Mathematics*, 61, 700 (1939). It is too early to predict whether it will be widely used by others.

¹ Vessiot, E., *Bulletin des Sciences Mathématiques*, 60, 257 (1936).

² This term was used with a different meaning in group theory by R. Dedekind in *Math. Annalen*, 48, 348 (1897).

SUBGROUPS TRANSFORMED ACCORDING TO A GROUP OF PRIME ORDER

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A necessary and sufficient condition that all the non-invariant subgroups of a given group G are transformed under G according to a group of prime order is that the norm or character subgroup of G is of prime index under G . When this index is 2 the character subgroup of G is known to be abelian¹ but when it exceeds 2 the character subgroup of G is either abelian or hamiltonian. In the former case the character subgroup of G is an arbitrary abelian group which involves at least two independent generators whose orders are powers of 2, such that at least one

of them has an order which is at least as large as 4, and G is the direct product of a non-abelian group whose order is a power of 2 and an arbitrary abelian group whose order is odd. Hence this case is practically reduced to the study of a special class of non-abelian groups of order 2^m which separately contain an abelian subgroup of index 2.

While all the non-invariant subgroups of each group in this special class are transformed under the group according to a permutation group of order 2, and there is an infinite number of distinct groups which corresponds to an arbitrary such given group of transformations of the non-invariant subgroups contained in G , the determination of all such possible groups requires detailed considerations which may not be of great interest. The special cases when this permutation group is of degree 2 or 4 have been considered and are obviously the simplest. The consideration of the permutation group of the transformations of all the non-invariant subgroups of G when these subgroups are transformed successively by all the different operators of G may serve as a convenient method of classifying all these groups.

When the character subgroup of G is of odd prime index under G the order of this subgroup must be divisible by the square of this odd prime number p instead of by its cube, which is the case when $p = 2$, as noted above. Since a hamiltonian, as well as an abelian group, is the direct product of its Sylow subgroups and every operator of G which is not contained in the character subgroup of G generates an invariant cyclic subgroup, it results directly that when this character subgroup is of index p under G the commutator subgroup of G must have an order which is equal to this prime number. Hence G must be the direct product of its Sylow subgroups when p is odd as well as when the index of its character subgroup is equal to 2. While in the latter case only one of these Sylow subgroups can be non-abelian, two of them may be non-abelian in the former case. When two of these Sylow subgroups whose direct product is equal to G are non-abelian each of them has a commutator subgroup of prime order.

From the preceding paragraph it follows that when the character subgroup of G is of prime index the determination of G is practically reduced to the determination of G when its order is the power of a prime number. In all of these cases all the non-invariant subgroups of G are transformed under G according to a permutation group whose order is equal to the index of this character subgroup under G and it is possible to construct G in such a way that the degree of this permutation group is an arbitrary multiple of its order. The simplest case presents itself when this permutation group is regular and hence G is conformal to the direct product of a cyclic group whose order is a power of a prime number and the group whose order is this prime number, according to a known theorem.

The group of lowest order which has a character subgroup of index 2 under the group is the group of order 16 which is obtained by extending the cyclic group of order 8 by an operator of order 2, which transforms into its fifth power every operator of this cyclic group, and there is only one group of order 16 which satisfies the condition that its character subgroup is of index 2 under the group. This results directly from the facts that such a group contains an abelian subgroup of index 2 and that each of its remaining operators generates an invariant subgroup which involves the commutator subgroup of order 2 of the group but does not transform into itself every subgroup of the group. The group of lowest order which has a character subgroup of odd prime index under the group is the non-abelian group of order p^3 which involves operators of order p^2 . There is obviously only one such group of order p^3 .

The preceding paragraph may serve to illustrate the following general theorem relating to the character subgroups of a given group G . *If the character subgroup of the group G is of prime index under G then the order of each of the operators of G which does not appear in its character subgroup is divisible by a higher power of this prime number than that of any of the operators of the character subgroup of G .* A proof of this theorem follows almost directly from the facts that the commutator subgroup of G is of prime order and that each of the operators of G which is not contained in the character subgroup of G generates an invariant subgroup of G . At least one of the operators of G which does not appear in the character subgroup of G has an order which is a power of the prime number which is the index of the character subgroup of G since this subgroup is an invariant subgroup of G . Moreover, every operator of G which does not appear in the character subgroup of G is divisible by the same highest power of the prime index of the character subgroup of G .

It should be noted that while a group may have many invariant subgroups it can have only one character subgroup. In view of the simple definition of the character subgroup and its usefulness in the study of certain groups, the question naturally arises why this subgroup did not receive a special name in the early development of group theory. This seems to be at least partly due to the fact that the early development of group theory was largely inspired by the usefulness of this subject in the theory of the solution of algebraic equations and in this theory the simple groups play a fundamental rôle. The character subgroup of a simple group of composite order is obviously the identity and hence character subgroups do not play an important role in the theory of simple groups of composite orders. Moreover, the character subgroups do not play a fundamental rôle in the study of the permutation groups of low degrees which received special attention in the early developments of group theory.

The smallest degree for which there exists a permutation group which has a character subgroup of prime index is 8 and only one of the two hundred groups of this degree has a character subgroup of prime index. This is the transitive representation on the smallest possible number of letters of the group of order 16 noted above. There is also only one group of degree 9 which has the property that it contains a character subgroup of prime index. This is the group of order 27 noted above when it is represented as a permutation group on the smallest possible number of letters. These statements, which can readily be verified, may serve to explain why the character subgroups failed to receive much attention in the early development of group theory, notwithstanding the simple definition of these subgroups and their interesting properties, especially when they are of small index.

The study of the groups whose character subgroups are of composite index is somewhat more difficult than the study of those whose character subgroups are of prime index, but the study of the former can often be reduced to a study of the latter types of groups. When the character subgroup is of composite index it cannot always be assumed that the commutator subgroup is of prime order and this naturally increases the difficulty of considering all the possible cases that may present themselves. If the order of a dihedral group is divisible by 4 its character subgroup is of order 2 but all other dihedral groups have the identity for a character subgroup. The fact that in this theorem the term central may be substituted for the term character subgroup suggests the close relations between the concepts of central and character subgroup. The former of these subgroups is obviously always included in the latter, but it cannot be of prime index, since the central quotient group is always non-cyclic.

¹ This fact can easily be derived from a theorem by R. Baer, *Compositio Math.*, **1**, 267 (1934). It was explicitly stated later by the present writer in these *PROCEEDINGS*, **29**, 240, (1943).

ON CONVEX TOPOLOGICAL LINEAR SPACES

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In an earlier note¹ the author introduced and discussed the notion of linear system. It is the purpose of the present note to apply this notion to the study of convex topological linear spaces.² Let X be an abstract linear space. Let X^* be the space of all linear functionals defined on X . With each convex topology t in X we associate the subspace L of X^* consisting of those linear functionals continuous with respect to t . In general, there will be many t 's associated with a single L and we obtain in this way a natural many-to-one correspondence between convex topological linear spaces and linear systems. It is our purpose here to correlate the properties of a convex topological linear space with those of its linear system and with the strength of its topology relative to that of the other convex topological linear spaces associated with the same linear system.

Our principal tool is a reformulation of von Neumann's³ observation that the topology of a convex topological linear space may be described by means of pseudo-norms. This reformulation, whose proof is a consequence of Fichtenholz's⁴ theorem on the relationship between norms and linear functionals, is as follows. Let L be an arbitrary total⁵ subspace of X^* . Let \mathfrak{F} be any family of pseudo-norm sets which has the following three properties. (a) For each two members of \mathfrak{F} there is a third member of \mathfrak{F} which contains them both. (b) If a pseudo-norm set is contained in a member of \mathfrak{F} then it is itself a member of \mathfrak{F} . (c) The set theoretic union of all members of \mathfrak{F} is L . Then there is a unique convex topology in X whose continuous linear functionals are precisely the members of \mathfrak{F} . Conversely, every convex topology in X associated with L may be obtained in this way. In other words, there is a natural one-to-one correspondence between the convex topologies associated with X_L and the "ideals of pseudo-norm sets which span L ."

As will be proved in the author's forthcoming paper on linear systems, every finite dimensional subspace of an X^* is a pseudo-norm set and the linear union of any two pseudo-norm sets is again a pseudo-norm set. Thus for any L the family of all pseudo-norm sets in L and the family of all finite dimensional pseudo-norm sets in L are both ideals which span L . As an immediate consequence we have:

THEOREM 1. *Let L be an arbitrary total subspace of X^* . Then the family of convex topologies in X associated with L not only is not empty but also contains a weakest member and a strongest member.*

This theorem suggests the following definitions. A *relatively weak* (*relatively strong*) convex topological linear space is a convex topological linear space which has a weaker (stronger) topology than any other such space with the same linear system. Normed linear spaces in their weak topologies are relatively weak convex topological linear spaces and in their norm topologies are relatively strong convex topological linear spaces. Because of the latter fact one can regard the notion of relatively strong convex topological linear space as a natural generalization of that of normed linear space. Theorem 3 below is of interest in this connection. In general, of course, a convex topological linear space will be neither relatively weak nor relatively strong but on the other hand, may be both.

The standard notion of boundedness⁶ in topological linear spaces coincides in the convex case with the boundedness for linear systems introduced in "IDS." That is, if X is a convex topological linear space and L is its family of continuous linear functionals then a subset A of X is bounded if and only if $\text{l.u.b.}_{(x \in A)} |l(x)| < \infty$ for each l in L . This has as an immediate consequence the fact that two convex topologies in X generate the same bounded sets if and only if their families of continuous linear functionals have identical bounded closures. Thus it is clear that not only are there, in general, many convex topologies with the same continuous linear functionals but also many convex topologies with different continuous linear functionals and the same bounded sets. These considerations lead at once to a proof of the following theorem and hence show that the converse of a certain theorem of Wehausen⁷ is not true.

THEOREM 2. *Let X be a convex topological linear space. Then every linear transformation from X to another convex topological linear space which takes bounded sets into bounded sets is continuous if and only if X is relatively strong and has a boundedly closed linear system.*

It follows from Theorem 2 and a slight extension of a theorem of Wehausen⁸ that every metrizable convex topological linear space is relatively strong and has a boundedly closed linear system. The question as to which boundedly closed linear systems are such that their associated relatively strong convex topological linear spaces are metrizable is answered at once by the Birkhoff-Kakutani⁹ group metrizability criterion and we have:

THEOREM 3. *Let X be a convex topological linear space and let X_L be its linear system. Then X is metrizable if and only if it is relatively strong and L is the union of an ascending sequence of pseudo-norm sets. (Such an L is automatically boundedly closed.)*

Thus given a linear space X there is a natural one-to-one correspondence between the metrizable convex topologies in X and the ascending sequences of pseudo-norm sets with total unions. It follows from a theorem in the theory of linear systems that such a union is a norm set if and only if all members past a certain one are identical. Thus the metrizable but non-

normable convex topologies correspond to the strictly ascending sequences. Using simple theorems about pseudo-norm sets it is possible to construct many examples of such sequences.

As a consequence of Theorem 3 one may prove:

THEOREM 4. *A relatively weak convex topological linear space is normable if and only if it is finite dimensional and is metrizable if and only if it is isomorphic to a subspace of the space (s) of Banach.¹⁰*

A topological linear space X , being a topological group, has a natural uniform structure.¹¹ Hence one may speak of its totally bounded subsets, its Cauchy directed systems, and of whether or not it is complete. It turns out that completeness in this sense is relatively rare and various authors have introduced several weaker notions which we shall now formulate. X is C_4 complete if it is complete as a uniform structure with respect to the convergence of directed systems. X is C_3 complete if every closed and bounded subset is C_4 complete. X is C_2 complete if every closed and totally bounded subset is C_4 complete. X is C_1 complete if it is complete as a uniform structure with respect to the convergence of sequences. X is T_2 complete if every closed and totally bounded subset is bicomact. X is T_1 complete if every closed and totally bounded subset is compact. It is more or less obvious that for $i = 2, 3$ or 4 , C_i completeness implies C_{i-1} completeness and that T_2 completeness implies T_1 completeness. It follows from the generalization to uniform structures of a well-known theorem on metric spaces¹² that C_2 completeness and T_2 completeness are equivalent. Finally von Neumann⁸ has shown that T_1 completeness implies C_1 completeness. Thus the five possibly distinct notions of completeness among those described above may be arranged in order so that each is weaker than or equivalent to its predecessor.

The principal theorems relating the completeness of convex topological linear spaces to their relative strength and to the properties of their linear systems are as follows.

THEOREM 5. *If $i = 1, 2$ or 3 and X is a C_i complete convex topological linear space then X is C_i complete in every relatively stronger convex topology.*

THEOREM 6. *If X is a C_1 complete convex topological linear space then the linear system of X is a complete linear system.*

THEOREM 7. *If X_L is a complete linear system whose conjugate system is boundedly closed then every convex topological linear space associated with X_L is C_3 complete.*

It is not known whether or not the converse of Theorem 7 is true. However, the following partial converse may be proved.

THEOREM 8. *If X_L is a regular linear system whose associated relatively weak convex topological linear space is C_3 complete and if X_L is relatively bounded then the conjugate of X_L is boundedly closed.*

*It is interesting to note that the truth of the strict converse of Theorem 7 would imply that every linear system of the form X^*_x is boundedly closed and hence answer the measure theory question of Ulam mentioned in "IDS."*

It is an obvious consequence of the definition that the linear system of a normed linear space is relatively bounded. Furthermore, it is readily verified that a normed linear space is reflexive if and only if its linear system is complete and has a boundedly closed conjugate. Finally, for relatively weak convex topological linear spaces, bounded subsets and totally bounded subsets are identical¹³ so that C_3 completeness and T_2 completeness are equivalent. Thus Theorems 7 and 8 have the following known corollary.

THEOREM 9. *For a normed linear space X the following are equivalent: (a) X is reflexive. (b) X is C_3 complete in its weak topology. (c) X is T_2 complete in its weak topology.*

Wehausen¹⁴ has pointed out that a T_1 complete topological linear space need not be of the second category. The first statement of the following combined with Theorem 7 shows that this may be extended to C_3 completeness and furnishes a wide class of examples including Wehausen's.

THEOREM 10. *If a convex topological linear space is of the second category then it is relatively strong and its linear system is uniform.*

One may also prove:

THEOREM 11. *If X is a convex topological linear space of the second category whose linear system is almost relatively bounded then X is normable.*

¹ Mackey, G. W., "On Infinite Dimensional Linear Spaces," these PROCEEDINGS, 29, 216 (1943). In the sequel we shall refer to this paper as "IDS" and shall use the definitions and notations introduced in it without comment or explanation.

² By a topological linear space we mean a real linear space which is at the same time a T_1 space in the sense of Alexandroff and Hopf (*Topologie I*, J. Springer, Berlin, 1935) and in which the topology is related to the algebra in such a manner that the operations of addition and multiplication by reals are continuous in both variables together. By a convex topological linear space we mean a topological linear space in which every point has a complete system of neighborhoods each of which is a convex set. These notions have been introduced in slightly different ways by various authors. See Wehausen, J. V., "Transformations in Linear Topological Spaces," *Duke Math. Jour.*, 4, 157 (1938), for a discussion. Also see Whitney, H., "Tensor Products of Abelian Groups," *Ibid.*, 5, 518 (1939), footnote 22, for a discussion of a popular misconception.

³ von Neumann, J., "On Complete Topological Spaces," *Trans. Amer. Math. Soc.*, 37, 1 (1935).

⁴ Fichtenholz, G., "Sur les fonctionelles linéaires, continues au sens généralisé," *Rec. Math. (Mat. Sbornik)*, N. S., 4, 192 (1938).

⁵ Wehausen has shown that the family of continuous linear functionals on a convex topological linear space X is always total; that is, for each non-zero member of X there is a continuous linear functional which does not take it into zero, loc. cit., Theorem 8.

⁶ See Hyers, D., "A Note on Linear Topological Spaces," *Bull. Amer. Math. Soc.*, 44, 76 (1938), for statements of the two standard definitions of boundedness and a proof of their equivalence.

⁷ Loc. cit., Theorem 2.

⁸ Loc. cit., Theorem 3'.

⁹ Birkhoff, G., "A Note on Topological Groups," *Comp. Math.*, 3, 427 (1936); Kakutani, S., "Ueber die Metrizierung der topologische Gruppen," *Proc. Im. Ac. Jap.*, 12, 82 (1936).

¹⁰ Banach, S., *Théorie des opérations linéaires* Warsaw, 1932, p. 10. By an isomorphism between two topological linear spaces we mean an algebraic isomorphism which is at the same time a homeomorphism.

¹¹ For a discussion of the notion of uniform structure see Weil, A., *Sur les espaces à structure uniforme et sur la topologie générale*, Hermann, Paris 1937.

¹² The theorem that a totally bounded metric space is complete if and only if it is compact. The generalization is essentially contained in G. Birkhoff's proof that C_1 completeness implies T_2 completeness. Birkhoff, G., "Moore-Smith Convergence in General Topology," *Ann. Math.*, 38, 39 (1937).

¹³ Various authors have proved this fact in special cases and the general case offers no new difficulties.

¹⁴ Loc. cit., Theorem 15.

NATURAL LOGARITHMS OF SMALL PRIME NUMBERS

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PART I. EXTENSION OF J. C. ADAMS' TABLE ABOVE LOG_e 7

In the summer of the year 1938 the author began the computation of the Napierian logarithms of 11, 13, 17, 19, 23, 29 and 31 with the object of extending J. C. Adams' basic 273-place table¹ which gives the logarithms of the four prime numbers falling between 1 and 11. In the following spring these calculations were interrupted intentionally in order to carry out a prerequisite investigation² of the perfection of the published records of Adams' constants. It was not until the early summer of the present year that the opportunity arose for effectuating the conviction that, aside from whatever intrinsic importance may attach to the project, the earlier sporadic work had progressed so far and had involved so much labor that it deserved to be revived, definitively checked and made accessible to other arithmeticians.

The calculations were usually performed with the aid of the formula $\ln(\alpha/\beta) = 2 \sum_{m=1}^{\infty} \{(\alpha - \beta)^{2m-1}(\alpha + \beta)^{-2m+1}(2m-1)^{-1}\}$. The composite numbers α and β were chosen to satisfy the simplifying condition $\alpha - \beta = 1$ and to contain as factors only the small prime numbers under consideration

In a few cases the expression 1 ± 10^{-p} yields numbers fulfilling the latter condition. The evaluation of $\ln(1 \pm 10^{-p})$ is relatively easy because it consists chiefly in the transcription of the reciprocals of small integers and these often have short recurring-decimal periods. For example, $\ln 11 = 2 \ln 10 - 2 \ln 3 + \ln(1 - 10^{-2})$.

The column headed T in table 1 gives the decimal place at which the particular approximation to $\ln N$ was terminated conformably to the condition that the error in the last figure should be numerically less than 4 units. For illustration, the logarithms of the first three entries in the column headed N were less than the respective more extended values

TABLE 1

N	α	$\beta = \alpha - 1$	T
11	9801 = $3^4 \cdot 11^2$	$2^3 \cdot 5^2 \cdot 7^2$	262
13	6656 = $2^9 \cdot 13$	$5 \cdot 11^3$	244
13	2432 = $2^7 \cdot 19$	$11 \cdot 13 \cdot 17$	262
19	6860 = $2^3 \cdot 5 \cdot 7^3$	19^3	300
23	10626 = $2 \cdot 3 \cdot 7 \cdot 11 \cdot 23$	$5^4 \cdot 17$	297
29	7425 = $3^3 \cdot 5^2 \cdot 11$	$2^3 \cdot 29$	302
29	12122 = $2 \cdot 11 \cdot 19 \cdot 29$	$17 \cdot 23 \cdot 31$	293
31	15625 = 5^6	$2^3 \cdot 3^2 \cdot 7 \cdot 31$	293

by 1.1, 2.9 and -3.5 units in the 262nd, 244th and 262nd decimal places. $\ln 17$ does not fit into this table. It was first given to at least 328 places in an earlier paper.²

The following independent calculations were made this year. $\ln 11$ to nearly 330 D from $\ln 2$, $\ln 3$, $\ln 5$ and $\ln(0.99)$ as formulated above. $\ln 13$ to 292 D from $\ln 2 + 2 \ln 3 + \ln 5 - \ln 7 + \ln(1.001) - \ln(0.99)$. The discrete terms required for the evaluation of $\ln(1.001)$ led at once to $\ln(0.999)$ and hence to 292 D of $\ln 37$, since $\ln 37 = 3 \ln 10 - 3 \ln 3 + \ln(0.999)$. Similarly $\ln 101$ was derived from $2 \ln 10 + \ln(1.01)$ to about 330 D .

An omnibus check on the logarithms of 2, 3, 5, 11, 17, 19, 23, 29 and 31 is afforded by the two approximations to $\ln 29$. These two values were identical as far as the 292nd decimal place, the value based upon $\alpha = 7425$ being greater than the other by 2.1 units of the 293rd place. In order to test simultaneously $\ln 7$, $\ln 13$ and $\ln 37$ the third one of these constants was computed from its equivalent $6 \ln 10 - 3 \ln 3 - \ln 7 - \ln 11 - \ln 13 + \ln(0.999999)$. The two calculations accidentally gave numbers which were identical throughout the 292 places of decimals covered in both cases. Since 290 decimals extend beyond 273 places by the wide margin of 17 figures, the practically perfect agreements in the cases of $\ln 29$ and $\ln 37$ constitute additional confirmation of my table^{2, 3} of terminal errors of J. C. Adams' results for $\ln 2$, $\ln 3$, $\ln 5$, $\ln 7$, $\ln 10$ and the modulus M . Inci-

dentially the natural logarithm of the prime 9901 was immediately available from the data underlying the second calculation of $\ln 37$. More specifically $\ln 9901 = 6 \ln 10 + \ln(1 + 10^{-6}) - \ln 101$ and $\ln(1 + 10^{-6})$ is obtained as the difference between two positive sums of series terms which when added give $-\ln(1 - 10^{-6})$, i.e., $-\ln(0.999999)$. The value of $-\ln(1 - 10^{-6})$ was found to be perfectly accurate through the 291st decimal place by forming the algebraic sum of the independently computed logarithms of $1 - 10^{-8}$ and $1 + 10^{-3}$ neither of which had been carried beyond the 292nd place.

Although table 2 was not designed to extend beyond the 290th decimal place, nevertheless, for sake of preservation, several of the constants are recorded in it to as many places as my original calculations justified.

TABLE 2

$\log_e 11 \equiv \ln 11 =$

2.39789	52727	98370	54406	19435	77965	12929	98217	06853	93741
71752	18567	70913	05736	23913	23671	30750	54708	00263	47914
14715	72588	81379	98522	25556	91585	95787	39535	53023	90801
10806	50516	41906	68067	50965	89460	66793	79382	46669	05466
38056	87286	99539	71661	60632	90270	01611	37003	06768	32876
13032	03053	18941	40170	57619	51443	38363	81829	78552	27496
92766	95044	70877	23320	84967	2869				

$\ln 13 =$

2.56494	93574	61536	73605	34874	41565	31860	48052	67944	76020
71164	19045	51066	34646	67324	41017	93995	74663	44048	94887
69258	19209	27627	21631	53215	44919	86587	01382	52681	16972
29775	17836	97080	47756	20441	04565	90117	93899	94342	73959
47399	27504	06138	96086	33400	55389	85298	09587	30794	11675
80366	50118	75546	48994	70418	20286	02681	41950		

$\ln 17 =$

2.83321	33440	56216	08024	95346	17873	12653	55882	03012	58574
47872	97237	73788	22925	75800	93128	09120	94868	03750	29475
18348	26204	71870	57291	39759	28419	46738	36429	97545	65742
02127	12599	13208	07209	04790	76471	68172	51666	60296	60850
69091	96813	96134	51492	95164	19209	44718	69393	25481	33184
68944	45037	58003	15646	02993	05896	37270	00327	36297	59273
99414	82424	46984	21556	64224	3439				

$\ln 19 =$

2.94443	89791	66440	46000	90274	31887	85353	72373	79261	29912
88185	37960	23640	92927	02064	19728	87141	58383	81573	98957
97040	63322	07501	36349	02195	37906	81320	61126	46333	28537
15643	14166	98613	78187	87130	14062	00072	09672	70164	72189
06225	84808	84881	47724	01849	91065	39837	74424	92535	66419
86124	83788	38520	86860	77295	97915	90976	72380	24863	1596

TABLE 2 (Continued)

ln 23 =

3.13549	42159	29149	69080	67528	31810	19611	84423	80314	84043
57419	98635	37748	29932	45984	79829	81984	01092	15299	48143
54197	21357	13301	36895	85872	86350	36337	80457	54969	57964
27532	98507	41826	31471	88086	06985	95650	61305	14549	41206
81850	53963	76031	51205	97403	56185	50700	45138	07926	99439
27302	39218	40357	55913	02511	03374	67096	48063	03899	

ln 29 =

3.36729	58299	86474	02718	32720	32361	91160	54945	12913	92274
40789	21670	35164	27807	81137	85233	32933	67114	81785	64226
45999	58472	51668	34726	55830	33111	79184	60754	32745	76975
61925	88548	65592	25938	94492	40291	26511	57790	06601	61839
73585	63417	83877	16117	90912	19923	09932	91700	80618	18630
13176	93093	61830	08901	07554	57942	25974	60636	88146	13706

ln 31 =

3.43398	72044	85146	24592	91643	24542	35721	04499	38930	48059
19717	56718	07247	49814	16597	55123	22138	64831	33608	66305
72976	93688	90398	44814	87054	32143	52712	99977	68643	49161
27045	52202	66130	63121	62119	28859	88191	47468	78202	92206
43825	83218	01055	61369	93182	02665	76228	52163	51765	75119
76862	66435	79840	93734	27098	77925	30215	88461	86	

ln 37 =

3.61091	79126	44224	44436	80956	71031	44716	39000	77587	16763
61636	44912	68119	29897	46990	36106	53990	21533	67216	86607
81808	95229	08956	67385	78157	92984	43954	74651	02120	68891
02611	49981	41622	16423	03807	02308	92316	27251	77371	33653
19855	41541	19834	17631	03772	77745	81986	49879	57152	94877
21273	35288	47290	44554	96265	31841	23252	72474	8	

ln 101 =

4.61512	05168	41259	45088	41982	66912	98915	68908	82587	19760
47499	31265	36170	20118	83602	34387	15046	80106	74195	67578
48461	17325	84189	82054	88979	84536	53314	82458	11107	28164
12623	54273	61284	07024	05871	00411	92814	54233	60460	35324
17599	37030	44195	97241	34826	95594	65644	43722	24317	35071
78641	95753	20437	80739	43466	00030	93398	27531	62861	66884
88607	84524	44510	30865	58470	3058				

ln 9901 =

9.20039	10411	22514	65355	70835	44526	72942	18823	93154	09871
76301	89336	86473	84353	18389	98176	50449	86829	04600	48200
75254	72631	95266	21899	27347	09888	76345	91607	13139	88680
41088	80615	98075	72570	26143	81908	47871	87781	20317	77635
35321	97831	24054	47251	97838	17483	74253	70759	56788	10327
13311	20502	38127	94051	35954	27321	84514	76001	17826	06029

PART II. SUPPLEMENT TO TABLE 4 OF THE MONOGRAPH⁴ ON 137-PLACE
VALUES OF $\text{LN}(1 \pm n \cdot 10^{-p})$

At the time of preparing the manuscript of the monograph in question the author finally had at his disposal two blank pages and these were then filled out completely with a collection of unpublished natural logarithms of his own computation. No attempt at tabular completeness was either attempted or professed. Recently while investigating the errors in Parkhurst's⁵ 100-place tables of common logarithms it was noticed that an excellent check⁶ on the accuracy of the non-terminal portions of a complete table of logarithms of integers is afforded by comparison of the sum of the logarithms with the factorial of the greatest value of N involved. Considerations of the kind just suggested found concrete expression in the calculation to more than 155 decimal places of the Napierian logarithms which are required for the completion of table 4 of the monograph for prime numbers lying between 1 and 101. The corresponding values of N are: 41, 43, 59, 61, 67, 73, 79, 83 and 89. Since the logarithms given in table 4 were not prepared originally for a 155-place table it was also necessary to extend to a greater number of decimals the values printed in the monograph for N equal to 47, 53 and 97. The results of this recent work are presented herewith in table 3. For sake of completeness and for the convenience of the user the values of $\ln 47$, $\ln 53$, $\ln 71$, $\ln 97$ and $\ln(100!)$ have been transcribed in extenso in this table.

The value of $\ln(100!)$ was computed from the 25 natural logarithms of the prime numbers lying between 1 and 101 in conformity with the exponents of the product $2^{97} \cdot 3^{48} \cdot 5^{24} \cdot 7^{16} \cdot 11^9 \cdot 13^7 \cdot 17^5 \cdot 19^5 \cdot 23^4 \cdot 29^3 \cdot 31^3 \cdot 37^2 \cdot 41^2 \cdot 43^2 \cdot 47^2 \cdot 53 \cdot 59 \cdot 61 \cdot 67 \cdot 71 \cdot 73 \cdot 79 \cdot 83 \cdot 89 \cdot 97$ which is the equivalent of $100!$. For comparison the previously published⁷ master value of $\ln(100!)$, as obtained from Stirling's series, was extended to about 165 decimal places. The value of $\ln(100!)$ calculated from the 25 logarithms was greater than the series result by about 2 units in the 158th place of decimals. Consequently the author considers it quite justifiable to claim absolute freedom from error for the 155-place table of natural logarithms of primes falling between 7 and 101 which is fully covered by tables 2 and 3.

TABLE 3

 $\ln 41 =$

3.71357	20667	04307	80386	67633	73037	40758	83764	10469	39930
16336	19262	91025	99786	16405	65750	59623	17141	37198	68110
37990	48939	53744	91846	75063	11642	35906	03925	40115	92439
75910	50								

 $\ln 43$

3.76120	01156	93562	42347	28425	13345	84703	55591	36184	88155
54151	91685	26492	28591	73872	98643	85375	91998	08399	72808
97505	68036	80967	91547	75955	03819	10483	62058	12917	26532
62783	P								

TABLE 3 (Continued)

ln 47 =

3.85014	76017	10058	58682	09506	69772	17370	88960	50502	02022
40332	00508	34680	68182	13505	80107	26106	89753	43474	59809
56451	57390	10175	10729	03275	70616	43115	00960	58517	33929
58279	P								

ln 53 =

3.97029	19135	52121	83414	44691	39029	05777	03599	77752	91121
76030	48129	47001	80046	33943	48985	85346	59944	48592	12298
42113	82048	10908	63018	33621	00776	77757	11087	88029	39899
16368									

ln 59 =

4.07753	74439	05719	45061	60503	73719	69762	40633	46789	33045
45295	12036	69705	92001	14265	42747	73593	39959	80263	37690
10443	87955	58849	62283	05125	71065	19560	03151	84719	48362
88905	n								

ln 61 =

4.11087	38641	73311	24875	13891	03425	61474	63156	81743	08126
10629	37383	64641	94398	06844	94873	11854	29206	10010	37730
23472	89224	26325	70597	96525	98303	37809	50939	53274	77279
59939	n								

ln 67 =

4.20469	26193	90966	05967	00719	96363	72275	05669	32903	22189
53371	37784	13077	52685	05528	08689	66389	14187	82102	54845
33962	19126	46600	14196	98720	64833	27536	36883	18077	54918
04238	P								

ln 71 =

4.26267	98770	41315	42132	94545	32513	03409	67595	76526	71056
61081	21425	80202	73515	06824	23036	59662	43324	27263	51335
40946	35034	57211	12385	45755	79963	96882	06266	76523	92546
74924	n								

ln 73 =

4.29045	94411	48391	12909	21088	57438	54257	09047	52844	87159
76645	95698	85716	17899	75920	59729	32763	29322	83485	88942
54031	42440	13917	21742	12010	86046	87538	49949	37814	99651
77229	N								

ln 79 =

4.36944	78524	67021	49417	29455	41481	41092	21735	41224	42260
96254	12171	11755	98060	61124	43227	81459	40365	77407	96066
38302	81749	58346	52592	14701	54346	68294	97850	73552	19750
82630	P								

ln 83 =

4.41884	06077	96597	92347	54722	23291	37045	30293	13056	66323
63701	87943	46293	85789	89888	99060	58384	27296	97057	34342
27952	73806	34506	29079	70222	33564	85011	83150	08277	05085
54550	N								

$\ln 89 =$

4.48863	63697	32139	83831	78155	40669	84921	94046	60387	13295
93641	06697	57728	79538	92779	45624	64470	63551	94947	57430
17288	16622	67819	47143	10095	03443	88022	22658	76701	87412
10057	<i>n</i>								

 $\ln 97 =$

4.57471	09785	03382	82211	67216	21703	96171	38089	14902	65878
13559	76234	36876	01772	91778	18712	53422	82745	03427	45499
26950	68064	83797	06627	53799	13535	58959	69188	44527	30295
78413	<i>n</i>								

 $\ln (100!) =$

363.73937	55555	63490	14407	99933	69655	63802	78239	21062	88727
47276	79448	87677	59444	47979	01991	41010	00241	97254	93196
15773	55972	29305	31198	01503	48915	04259	44052	15183	63651
21393	39800	973	<i>N</i>						

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⁴ Uhler, H. S., *Original Tables to 137 Decimal Places . . .*, New Haven (1942).

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⁷ Uhler, H. S., *Proc. Nat. Acad. Sci.*, **28**, 59-62 (1942).

NOTICE. Owing to the preoccupation of members of the NATIONAL ACADEMY OF SCIENCES and of the NATIONAL RESEARCH COUNCIL with scientific aspects of the war effort, the manuscripts available for the October issue were so few that they were held over for this combined October-November number. It is not unlikely that from time to time similar combinations of numbers may be advisable, and it may not be possible always to give advance notice thereof.

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GENIC INDUCTION OF AN INHERITED CYTOPLASMIC DIFFERENCE

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Although many biologists consider the genes to be the sole determiners of heredity, there are those who feel that the cytoplasm contains a system of independent entities which in some cases controls the expression of certain characteristics. The terms genome and plasmone have been used to denote the system of genes and of cytoplasmic entities, respectively. Although the development of chlorophyll has been shown in hundreds of cases to be under genic control, there are a number of instances where chlorophyll variegation is inherited independently of the genome. These chlorophyll variegations, transmitted through the female line only, constitute the most compelling evidence for cytoplasmic inheritance. In these cases the physical entities in the cytoplasm are known to be the plastids; in other cases of plasmatic inheritance the nature of the entities in the cytoplasm can only be conjectured.

In maize there are more than one hundred cases where the development of chlorophyll is under genic control. Two examples of cytoplasmically inherited chlorophyll variegation have been reported,^{1, 2} as has one case of the cytoplasmic inheritance of male sterility.³ Among the chlorophyll characters in maize which are genically controlled is that of *iojap*. Maize plants homozygous for the recessive gene *iojap* (*ij*) exhibit a chlorophyll striping or variegation.⁴ Considerable variation is found in the extent and pattern of the green and white areas of the leaves and culm. The *ij* gene is situated in chromosome 7; its position in the linkage group has been determined with some precision through 3-point linkage tests.⁵

When *ij ij* plants are used as the pollen parent in crosses with normal (*Ij Ij*) individuals, the F_1 progenies consist wholly of green plants—i.e., the *ij* allele is completely recessive to the normal *Ij* allele. However, as Jenkins first noted, when *ij ij* individuals are used as the female parent

in crosses with *Ij Ij* plants, the F_1 progenies often contain white and/or striped seedlings in addition to the expected green ones. Great variations are found in the proportions of white and striped seedlings; sometimes all the seedlings from an F_1 ear are white. (The *ij* plants used in these experiments arose in the F_2 generation of the cross $Ij \text{ } \varnothing \times ij \text{ } \sigma$.)

A cytological examination of the white regions of *ij ij* plants disclosed that the plastids of the mesophyll cells not only lacked chlorophyll but also were much smaller than were the plastids of the normal green areas. It appears, therefore, that the *ij* gene is able to induce a modification in the plastid. That these modified plastids have a genetic continuity, which is not affected by the genic constitution and therefore may be considered as mutations is suggested by the observations that the F_1 plants of *Ij ij* constitution may be white or striped. If a white F_1 seedling arises, it is assumed that the cytoplasm of the egg cell carried only plastid primordia of the mutated type, while striped individuals are assumed to have come from an egg cell whose cytoplasm contained both normal and mutated plastid primordia, with somatic segregation of the two plastid types giving the striped pattern. Support for the above hypothesis was obtained from a cytological examination of the white areas of *Ij ij* plants which showed them to have a type of plastid similar to that found in the white areas of the parent *ij ij* plant. Furthermore, both types of plastids were found in certain green cells, adjacent to the white regions.

The correctness of the hypothesis that in *ij ij* plants an irreversible mutation is induced in the plastid by the *ij* allele is proved by the following evidence. Striped F_1 plants from the cross $ij \text{ } \varnothing \times Ij \text{ } \sigma$ were crossed with pollen from unrelated *Ij Ij* individuals. The backcross progenies were obtained from such crosses. Genetically each population consisted of equal numbers of *Ij Ij* and *Ij ij* plants. Some of the backcrossed ears on F_1 striped plants gave progenies consisting entirely of green seedlings. Such ears, it is argued, arose from green sectors of the F_1 plants and hence the egg cytoplasm carried normal plastid primordia. Other ears gave rise to progenies with varying percentages of green, striped and white seedlings. Here it is assumed that the ears came from both green and white tissue and therefore some egg cells had normal plastids while some had mutated plastid primordia. Occasionally all the progeny from a back-crossed ear consisted of white seedlings only and consequently came from a white sector of the parent plant. These all white populations are the most instructive. Half of the individuals in such populations are homozygous for the *Ij* allele and half are heterozygous. It is a pertinent fact that in *Ij Ij* cells the mutant plastid continues to give rise to mutant plastids; there is no control by nuclear factors on the type of plastid. Although induced by a nuclear factor,

the *ij* gene, the mutated plastid, like a Frankenstein monster, is no longer under the control of its maker.

The data given here are of direct interest in connection with the nucleus-plasma problem, but they may have some significance in the field of both normal and abnormal growth and differentiation. The basis of cellular differentiation is one of the great problems of biology. All nuclei of an organism presumably have the same genic constitution, and yet morphological and physiological differences arise. That the maintenance of these differences is not entirely determined by the differing local conditions is shown by the persistence of certain specificities when cell multiplication of isolated differentiated cells occurs in tissue culture. The view that cellular differentiation is cytoplasmic seems to require that the cytoplasm contains elements of a hypothetical nature which are modified by interaction with nuclear products. In the case reported here a known constituent of the cytoplasm, the plastid, has been modified by a nuclear factor and is transmitted thereafter by cytoplasmic heredity.

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GENE AND CYTOPLASM. I. THE DETERMINATION AND INHERITANCE OF THE KILLER CHARACTER IN VARIETY 4 OF *PARAMECIUM AURELIA*¹

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The present paper reports a previously unknown system of relations between a gene and a cytoplasmic substance, both of which are required for the development of an hereditary character. When some of the cytoplasmic substance is present, the gene controls its continued production; but when the cytoplasmic substance is absent, the gene cannot initiate its production. Addition of the cytoplasmic substance to an organism, lacking the character dependent on it, but containing the required gene, results in the continued production of the substance, in the development of the character determined by the combined presence of gene and cytoplasmic substance, and in the hereditary maintenance of the character in successive generations.

The pair of characters whose determination and inheritance are to be analyzed are designated "killer" and "sensitive." They are found in diverse races of variety 4 of *Paramecium aurelia*. Of the four available races of this variety, only race 51 is a killer; the other three races (29, 32 and 47) are sensitive. Fluid in which the killer race has lived kills individuals of the sensitive races. The killing of sensitive animals is preceded by characteristic morphological aberrations, particularly by shifting of the posterior part of the body to the aboral side. The killer and sensitive characters are alternatives in inheritance and never exist together in the same individual. In practice, the character of an unknown clone is tested by mixing samples of it (1) with the killer race 51, and (2) with a sensitive race. If the characteristic abnormalities and corpses are produced in mixture (1), the unknown is sensitive; if produced in mixture (2), it is a killer. Sensitive animals begin to show abnormalities only after at least four hours of subjection to the killer fluid. It is possible to cross killers with sensitives if they begin to mate soon after they are brought together, and this is readily accomplished by bringing together opposite mating types when in sexually reactive condition. If the conjugant pairs are removed to fresh culture fluid soon after they unite and the two members of each pair are put into separate culture vessels soon after conjugation has been completed, the sensitive member of the pair is never injured by the contact with a killer during mating.

In the original races, the characters killer and sensitive are invariably inherited. All the vegetative and sexual progeny within race 51 are killers. All the vegetative and sexual progeny within the sensitive races are sensitive. Only the sensitive race 32 is reported upon in this paper; results with other sensitive races bring out certain further important points which will be set forth in the next paper of this series.

When the pure killer race 51 is crossed to the pure sensitive race 32, the two exconjugants of each pair produce phenotypically different clones: one is a killer and the other is sensitive. By marking the parents, it is readily demonstrated that the F_1 killer clones are those that derive their cytoplasm from the killer parent and the F_1 sensitive clones are those with cytoplasm from the sensitive parent. This result is totally unexpected and requires explanation because, as is well known, the nuclear processes during conjugation are such that the two mates, after reciprocal fertilization, have the same genotype and should produce clones alike in their hereditary characters. The following experiments were designed to provide the required explanation.

Experiment 1: Autogamy in F_1 Killers.—Ultimate genic control of the alternative characters is indicated by observations on the results of autogamy in one of the two classes of F_1 clones, the killers. Autog-

amy^{3, 4, 5} is a process in which identical, haploid, gamete nuclei in a single unmated cell unite to produce a diploid syncaryon from which all subsequent nuclei of the clone are derived. Hence all clones derived from autogamous individuals are necessarily homozygous and, when heterozygotes undergo autogamy, half become homozygous for one allele and half for the other. Of 306 F_1 killers that went through autogamy, 167 produced clones of killers and 139 produced clones of sensitives. The segregation ratio is reasonably close to the theoretical 1:1 ratio (deviation 14, standard error 8.7) and so indicates that the F_1 killers were heterozygous for a pair of alleles determining the alternative characters.

Experiments 2, 3, 4 and 5: Further Fertilizations within Each of the Two Classes Obtained in Experiment 1.—If the F_1 killers were heterozygotes, the two classes that segregated from them at autogamy (expt. 1) should be the two possible classes of homozygotes. Further breeding tests confirmed this: the sensitive F_2 clones yielded only sensitive progeny after further autogamies (expt. 2) and after conjugation with each other (expt. 3); the killer F_2 clones yielded only killer progeny after further autogamies (expt. 4) and after conjugation with each other (expt. 5). Hence the two classes of F_2 clones obtained in approximately equal numbers at autogamy in F_1 killers (expt. 1) are pure breeding or homozygous for the alternative characters. The genic determination of these characters was further tested in experiment 6.

Experiment 6: Crosses between Different Clones of F_1 Killers.—If the F_1 killers are heterozygotes, conjugation between two such clones should yield the usual F_2 ratio of 3:1 and should show which allele is dominant. From 443 pairs of conjugants the 652 clones from the two members of 326 pairs were killers and the 234 clones from the two members of 117 pairs were sensitives. This agreement with the theoretical 3:1 ratio (deviation $6\frac{1}{4}$, standard error 9) confirms the heterozygosity of the F_1 killers and shows that the killer gene (K) is dominant over its sensitive allele (k).

Since F_1 killers are heterozygous (K/k), their pure breeding parent races must have the two possible homozygous combinations: the killer race is K/K and the sensitive race is k/k . As the killer gene is dominant (expt. 6), all the F_1 should be heterozygous killers; but this condition has been demonstrated only for one of the two F_1 clones from each pair of hybrid exconjugants. The other is not a killer, but sensitive (see p. 330). Is the disagreement here merely phenotypic or genotypic also? Experiments 7 and 8 answer this question.

Experiment 7: Cross of F_1 Sensitives to F_1 Killers.—In order to discover the genotype of the F_1 sensitives, they were crossed to F_1 killers known (expts. 1-6) to be heterozygotes. From this cross were obtained 294 conjugant pairs yielding a killer clone from one member and a sensitive

clone from the other member of each pair: and 126 conjugant pairs yielding sensitive clones from both members of each pair. The meaning of this result is best brought out by considering first those clones that derived their cytoplasm from the F_1 killers, i.e., one member of each pair of exconjugant clones.

These cytoplasmic descendants of the F_1 killers included 294 killer clones and 126 sensitive clones. This is closer to a theoretical 3:1 ratio (deviation 21, standard error 8.9) than to any other simple genetic ratio. In the detailed paper to be published later it will be shown that the small discrepancy not due to sampling error is due to the occurrence, in a small percentage of the united pairs, of a process differing from normal conjugation. This process is essentially double autogamy or, as it has been called,⁶ cytogamy. In most crosses it can readily be detected and the data can be corrected for it; but, in crosses of the type under discussion, its detection is so laborious as to be quite impracticable.

The 3:1 ratio in this group shows that both parents were heterozygotes, as in crosses between F_1 killers (expt. 6). One parent was a clone of F_1 killers (known to be heterozygotic) and the other parent was a clone of F_1 sensitives. Hence, the F_1 sensitives must also have been heterozygotic (K/k) and this agrees with expectations from the breeding experiments 1 to 6. The disagreement with expectation is merely in their sensitive phenotype. The killer gene K , dominant in race 51 cytoplasm (expt. 6), is certainly not dominant in race 32 cytoplasm.

Further information on the behavior of gene K in race 32 cytoplasm is provided by consideration of the phenotypes of the other member of each pair of exconjugant clones produced in experiment 7, namely, those deriving their cytoplasm from the F_1 sensitives. All of the 420 clones of this group were sensitive. From the now known genotypes of their parents, $K/k \times K/k$, one-fourth of these 420 clones should be k/k and sensitive, one-half should be K/k and sensitive (because, as shown above, K is not dominant in race 32 cytoplasm) and one-fourth should be K/K . The fact that no killer clones were obtained in this group indicates that even the K/K clones were sensitive. In other words, the killer gene K is completely unable to produce the killer phenotype in race 32 cytoplasm. Experiments 8, 9 and 10 confirm this conclusion.

Experiment 8: Autogamy in F_1 Sensitive Clones.—As in the case of autogamy in heterozygous F_1 killer clones (expt. 1), autogamy in heterozygotic F_1 sensitive clones should yield K/K and k/k clones in a ratio of 1:1. But as the F_1 sensitive clones and their autogamous progeny derive their cytoplasm from race 32 and as the killer gene K is unable to produce the killer phenotype in this cytoplasm (expt. 7), both classes of exautogamous clones should be sensitive. In agreement with this, all of the 148 exautogamous clones from F_1 sensitives were sensitive.

Experiment 9: Crosses of F_2 Sensitive Clones (Obtained by Autogamy from F_1 Sensitives) to F_1 Killers.—It is practically impossible to test whether the 1:1 ratio predicted in experiment 8 is actually obtained because each clone would have to be separately tested for genotype by elaborate breeding experiments. More important than the ratio is the question of whether there are in fact produced, as predicted, sensitive clones homozygous for the killer gene. Therefore, only a few of the exautogamous sensitive clones from experiment 8 were fully tested to see if any had the predicted K/K genotype. The test consisted in crossing them to heterozygous F_1 killers. In this cross, any tested clone that contains the recessive gene k either in homozygous or heterozygous condition would yield some pairs in which both members produce sensitive clones; but if the tested clone is homozygous for the killer gene K , all the pairs of conjugants obtained in the cross to F_1 killers would yield a killer clone from one member of the pair and a sensitive clone from the other. Among the few clones tested, three gave the latter result. For example, 36 pairs were obtained from the cross of one clone to F_1 killers and from each pair there arose one clone of killers and one clone of sensitives. The other two were tested on an even larger scale and gave the same result. Hence, some of the exautogamous clones obtained from F_1 sensitive parents are homozygous for the killer gene though phenotypically sensitive. This confirms the conclusion drawn from experiment 7 that the killer gene is unable to determine the killer phenotype in race 32 cytoplasm even when it is present in homozygous condition.

Experiment 10: Sensitive Clones Homozygous for the Killer Gene Retested after the Passage of Several Months.—In order to exclude the possibility that the sensitivity of the K/K clones with race 32 cytoplasm was due either to the delayed action of the K gene or to the mutation of K to k in race 32 cytoplasm, these clones were retested at intervals of 2, 4 and 6 months. The tests showed that the sensitive phenotype and the K/K genotype were maintained. (The tests were the same as the one employed in experiment 9.) During the six months that these clones were cultured, many successive sexual generations must have occurred, for autogamies recur at intervals of 3 to 7 days in mass cultures of variety 4. Hence the gene K remains constant and is not only temporarily but permanently incapable of determining the killer phenotype in race 32 cytoplasm.

These results raise the question of cytoplasmic inheritance. In cytoplasm of race 51, the gene K determines the killer character; in cytoplasm of race 32, the same gene does not determine the killer character. This difference in the effect of gene K in different cytoplasms persists through many sexual generations, presumably without limit. Does this warrant the conclusion that the cytoplasms of the two races possess hereditary

differences that are independent of the genes? Experiment 11 answers this question.

Experiment 11: Cross of F_2 Sensitive Clones (Derived by Autogamy from F_1 Killers) to the Killer Race 51.—Experiments 1, 4 and 5 showed that half of the clones obtained by autogamy from F_1 killers were sensitive because they were homozygous for the sensitive gene k . The cytoplasm of these clones is derived from race 51. If the property of race 51 cytoplasm which permits the killer gene K to determine the killer character is inherited independently of the genes, reintroduction of gene K into these sensitive clones should result in the restoration of the killer character. The gene K was put back into them by mating them to the homozygous killer race 51; but the killer character failed to develop. In all of the 96 pairs of conjugants, the clone produced by the exconjugant deriving its cytoplasm *directly* from the killer parent remained a killer; but the clone produced by the exconjugant deriving its cytoplasm *indirectly* from race 51, through a sensitive F_2 clone, remained sensitive. Hence, when the gene K is replaced by its sensitive allele, k , the cytoplasm of race 51 becomes, like the cytoplasm of race 32, incapable of developing the killer phenotype when gene K is reintroduced into it. The property of race 51 cytoplasm which permits the killer gene to determine the killer character is thus not inherited independently of the genes, but is dependent on the uninterrupted presence of the gene K .

This experiment shows that the killer character depends on the combined presence of the killer gene K and something else. The other factor, when present, is reproduced under the influence of gene K and ceases to be reproduced when gene K is absent. Moreover, the failure of the killer character to develop in sensitive clones into which gene K has been introduced shows that gene K is unable to initiate the production of this other essential factor and that the latter is not carried over from one mate to the other during conjugation. In other words, it is not present in the "male," migratory, gamete nucleus at the time of fertilization. Experiments 12 and 13 were designed to throw light on the location within the cell of the essential factor other than gene K .

Experiment 12: Transfer of the Other Factor from one Cell to Another.—Under certain conditions, not yet entirely known,⁷ pairs of conjugants in variety 4 either remain united unduly long or permanently. At the normal time for separation of the mates, they separate everywhere except in the region of the paroral cones where a thin connecting band of cytoplasm appears. After prolonged union in this way, in some pairs the mates separate completely; but in others the band of union increases in width and the mates remain permanently united, though normal single animals are given off from the separated regions at the first few fissions. Such connections between mates provide opportunity for the transfer

of material from one to the other, especially in those pairs that develop a broad connecting band. In crosses between killers and sensitive clones with the killer gene *K*, when a cytoplasmic connection was established between the mates, the normal single animals produced from *both* of them yielded killer clones. Hence the formerly sensitive mate must have produced a clone with the killer phenotype and this result is correlated with the possession of the gene *K* plus temporary cytoplasmic continuity with a killer animal. In the same crosses, when separation of the mates occurred at the normal time, each mate remained phenotypically unchanged: one produced a clone of killers and the other a clone of sensitives. Hence the transformation of the sensitive into a killer clone in the cytoplasmically united pairs must have been due to the transfer from the killer to the sensitive mate of the material required in addition to gene *K* for the development of the killer phenotype. Moreover, of the gamete nuclei, syncaryon and derivatives of the syncaryon, only the migratory gamete nucleus goes across from one mate to the other in the cytoplasmically united pairs, and previous experiments have shown that the migratory nucleus does not carry the essential material with it; therefore this material at this time must be outside of these nuclei. The material could be in either the cytoplasm or the many pieces into which the old, disintegrating macronucleus has broken down, for these are both free to migrate from mate to mate across the broad cytoplasmic connecting band. There is, however, no known direct connection between the pieces of the old macronucleus and the new nuclei formed from the products of the syncaryon; and the old macronuclear pieces soon disappear, while the killer character is permanent and hereditary. Hence the essential material must be at least for a time outside the nuclei in the cytoplasm. There is as yet no evidence concerning the question as to whether it is ever in the nuclei. The substance whose continued production is controlled by gene *K* and whose presence is required for the development of the killer phenotype may therefore be designated as the killer cytoplasmic factor or substance. Whether this is the same as the substance that produces the killing action on sensitive cells or a precursor of it remains to be discovered.

Experiment 13: Demonstration of the Killer Cytoplasmic Factor in Cells That Have Just Lost the Killer Gene.—The killer cytoplasmic factor was shown in experiment 11 to disappear from cells that lose the killer gene *K*. However, only if this factor did not exist in the cytoplasm but was always indissolubly connected with gene *K* would its disappearance be expected to coincide exactly with the loss of gene *K*; otherwise, its disappearance should follow loss of gene *K* by an appreciable amount of time. If it could be detected in the cell for a considerable period after gene *K*

is replaced by k , this would provide further evidence for its cytoplasmic localization.

The method of detection employed was essentially the same as the one used in experiment 11: F_2 sensitives (k/k) that had arisen from F_1 killers (K/k) at autogamy were mated to the killer race 51 (K/K). In experiment 11 this cross was made several days after the genotype had changed at autogamy from K/k to k/k . At that time there was no evidence of the presence of the cytoplasmic factor, for return to the K/k genotype did not result in the development of the killer phenotype. In the present experiment, the same cross was made at intervals of only two to five fissions (one to two days) after the F_1 killer (K/k) had changed to an F_2 sensitive (k/k) at autogamy. Description of the laborious technique involved in bringing about conjugation so early in the history of a clone of known constitution will be reserved for the full paper to appear later.

Altogether 21 such F_2 sensitive clones were induced to conjugate with the killer race 51 within five fissions (2 days) after their origin at autogamy. Three of the four that conjugated two or three fissions after autogamy, four of the ten that conjugated three or four fissions after autogamy, and one of the seven that conjugated five fissions after autogamy, making a total of eight crosses, yielded clones of killers from *both* members of a pair of conjugants. Hence the sensitive parent, as well as the killer parent, must have yielded a clone of killers in these eight cases; and the cytoplasmic factor must still have been present. The data also indicate that the cytoplasmic factor is present in fewer and fewer cells with increase of time and number of fissions since loss of gene K , until, as shown in experiment 11, it has completely disappeared after a few days. The period during which the cytoplasmic factor remains after the gene for its production is removed corresponds closely to the period previously found^{8,9} for the "cytoplasmic lag" in change of phenotype following other changes in hereditary constitution in *P. aurelia*. Comparable gradual loss of a gene-controlled cytoplasmic factor may be involved in many or all such situations.

Both experiments 12 and 13 show: (1) that the factor required in addition to gene K for development of the killer phenotype is present in the cytoplasm of killer cells; (2) that gene K determines the continued production of this cytoplasmic factor, for sensitive cells converted into killers became permanently and hereditarily so; and (3) that this can be accomplished when the cytoplasmic factor is at least initially outside the nucleus in the cytoplasm. Both experiments also show (4) that there was present in one cell at least twice as much of the cytoplasmic factor as required by gene K to enable it to produce more. This was shown in experiment 12 by the fact that the killer member of the cyto-

plasmically united pairs provided enough of the cytoplasmic factor to enable genes in both cells of the pair to produce more; and in experiment 13 by the fact that in each of three different crosses of exautogamous clones to race 51, there were obtained *two* pairs of conjugants yielding killer clones from both members of the pair. Hence, in each of these three clones there was present after gene *K* was lost enough of the cytoplasmic factor so that it could be distributed to two daughter cells and still have in each enough to bring about the production of more cytoplasmic factor when gene *K* entered the cell.

The preceding 13 experiments provide the basic information required for discovery of the system of determination and inheritance of the killer and sensitive characters. Many other breeding experiments have been performed to test further the validity of the conclusions drawn in the preceding pages. Space does not permit a detailed account, but the crosses and observed results in some of the more important experiments are listed below.

Experiment 14.—Cross of F_1 killers (K/k) to race 32 (k/k); 210 pairs of conjugants. Result: 116 pairs yielded sensitive clones from both members of the pair; 94 pairs yielded a killer clone from one member and a sensitive clone from the other.

Experiment 15.—Cross of F_1 killers (K/k) to race 51 (K/K); 104 pairs of conjugants. Result: killer clone from each member of every pair.

Experiment 16.—Cross of F_1 sensitive (K/k) by F_1 sensitive (K/k); 431 pairs of conjugants. Result: all 862 clones sensitive.

Experiment 17.—Cross of F_1 sensitive (K/k) to race 32 (k/k); 200 pairs of conjugants. Result: all 400 clones sensitive.

Experiment 18.—Cross of F_1 sensitive (K/k) to race 51 (K/K); 107 pairs of conjugants. Result: in every pair, one member produced a sensitive clone and the other produced a killer clone.

In every experiment the observed results are those required by the conclusions drawn from the first 13 experiments.

The determination and inheritance of the alternative characters killer and sensitive in races 51 and 32 appear therefore to involve the following system. Killer depends upon the combined presence of the dominant gene *K* and a cytoplasmic substance. The continued production of this substance depends upon gene *K*; but gene *K* is unable to initiate its production when none is present. The alternative character sensitive invariably develops regardless of genic constitution when the cytoplasmic substance is absent. A recessive allele of *K* is unable to determine the continued production of the cytoplasmic substance even when some of it is present. All of the nine pairs of characters examined in five varieties of *P. aurelia* show the same peculiar division of the F_1 into two classes. This suggests that a comparable system of determination and inheritance

is widespread in this species. The significance of the system in relation to a number of problems of biology will be discussed in the next paper of this series.

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GENE AND CYTOPLASM. II. THE BEARING OF THE DETERMINATION AND INHERITANCE OF CHARACTERS IN *PARAMECIUM AURELIA* ON THE PROBLEMS OF CYTOPLASMIC INHERITANCE, PNEUMOCOCCUS TRANSFORMATIONS, MUTATIONS AND DEVELOPMENT¹

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The first paper³ of this series demonstrated that the character "killer" in variety 4 of *Paramecium aurelia* is dependent upon a cytoplasmic substance which normally fails to accompany the "male" gamete nucleus as it passes from one mate to the other during conjugation. The continued production of this determining cytoplasmic substance depends, however, on a dominant gene, *K*; replacement of *K* by its recessive allele, *k*, results in the disappearance of the active cytoplasmic substance. Nevertheless, the gene *K* is unable to initiate production of the cytoplasmic substance; introduction of *K* into a sensitive cell is not followed by development of the killer character. But if a non-killer (sensitive) cell containing gene *K* is supplied with some cytoplasm from a killer cell, or if a genotypically sensitive cell containing the cytoplasmic factor is supplied with gene *K*, the gene *K* controls the continued production of the killer cytoplasmic substance. This system of determination and inheritance appears to be typical for all characters in most varieties of *P. aurelia*. The preceding facts may have important applications to other fields of biology. The present paper attempts to point out some of these.

1. *Cytoplasmic Inheritance*.—Inheritance through the cytoplasm (aside from plastid inheritance) has been reported by a number of investigators

of mosses and flowering plants; this work has been critically reviewed by Correns⁴ and others. The observations on which the interpretation of cytoplasmic inheritance is based are closely parallel to some of those made on *Paramecium*. Full analysis shows that the interpretation in terms of cytoplasmic inheritance in *Paramecium* is an illusion and untenable. This raises the question as to whether the same interpretation based on similar evidence in plants is sound.

In plants the fundamental observations are: (1) reciprocal crosses yield different results; (2) the differences persist through subsequent generations in the female line of descent, even when all or nearly all of the genes of the female parent have been replaced by genes from the male parent.

Observations on *P. aurelia* precisely like the first one mentioned in the preceding paragraph were reported in the first paper³ of this series. Each pair of conjugants between diverse clones or races constitutes a pair of reciprocal crosses: One member of the pair of conjugants contains all the cytoplasm and one gamete nucleus from one parent and only a gamete nucleus from the other parent; the other member of the pair of conjugants has its cytoplasm and one gamete nucleus from the other parent and only a gamete nucleus from the first parent. These reciprocal crosses yield different results when the killer race is crossed to a sensitive race: the one in which the cytoplasm comes from the killer race produces a clone of killers and the one in which the cytoplasm comes from the sensitive race produces a clone of sensitives. Reciprocal crosses in *Paramecium*, as in plants, yield different results.

The parallel between the plants and *Paramecium* with respect to the second fundamental observation in the work on cytoplasmic inheritance is best shown by certain additional results not set forth in the first paper of this series. If, instead of using the sensitive race 32, reported upon in the previous paper, the sensitive race 47 is employed in crosses to the same killer race 51, the same results are obtained in the F_1 generation: reciprocal crosses give different results; those with cytoplasm from the killer race are killers and those with cytoplasm from the sensitive race are sensitive. But very different results follow in subsequent generations. The F_1 killers, though they have genes from both races, show no segregation of these traits in any breeding tests. In both autogamy and in backcrosses the progeny that derive their cytoplasm from the killer race remain killers. Likewise, the progeny of the F_1 sensitive clones that derive their cytoplasm from the sensitive race remain sensitive.

The preceding breeding experiments show, as do the comparable experiments with plants, that the hereditary differences under examination are due to cytoplasmic and not to genic differences. This was further demonstrated⁵ directly by providing an opportunity for transfer of cyto-

plasm from a killer to sensitive cell through a cytoplasmic connecting bridge between them. When cytoplasm of the killer race 51 gets in this way into sensitive animals of an appropriate genotype, the latter are transformed from the sensitive to the killer condition and the new character is inherited in all subsequent generations by those progeny that obtain their cytoplasm from the transformed individuals. This evidence makes the demonstration of the determinative influence of the cytoplasm complete. The persistence of the effect through the passage of generations would appear to justify the conclusion of cytoplasmic inheritance.

This conclusion would have been unavoidable if only races 47 and 51 had been available. Only the extremely good fortune of having the race 32 for use in further analysis prevented me from falling into this error. As set forth in the preceding paper,⁸ race 32 differs from race 51 not only in its cytoplasm (as does also race 47), but also in a gene which controls the cytoplasmic difference. When the killer gene *K* in race 51 is replaced by the sensitive allele *k* from race 32, the cytoplasmic factor determining the killer condition ceases to be reproduced. The phenomenon under analysis is thus not cytoplasmic inheritance, but continued production of a cytoplasmic substance under the influence of the single gene *K*. The false conclusion drawn from analyses limited to the sensitive race 47 is a consequence of the fact that this sensitive race, like the killer race 51, possesses the killer gene *K*. As previously shown,⁸ this gene cannot initiate production of the killer cytoplasmic substance, but merely controls its continued production when some is already present. The full proof, from a variety of experiments, that the race 47 contains the killer gene *K* will be given in a later paper. For the present, the facts already given above (absence of segregation in further breeding of *F*₁ killers) will suffice.

The preceding analysis shows that results such as those obtained with the plant material and with *Paramecium* (in the crosses not involving race 32) justify the conclusion that the differences observed in reciprocal crosses and subsequent generations are due to cytoplasmic and not to genic differences; they do not, however, justify the conclusion that cytoplasmic inheritance, in the sense of independent, self-multiplying, cytoplasmic determinants, is involved. The cytoplasmic differences may be perpetuated, not by independent cytoplasmic determinants, but by genes which are alike in the two forms crossed; and these genes, like the killer gene, may be unable to initiate production of the cytoplasmic substances involved. Only by means of specially favorable material can the genic control of the cytoplasmic substances be discovered.

2. *Environmental Control of Genetic Characters in Bacteria.*—The phenomena of the inheritance of the antigenic properties of *Pneumococcus* constitute one of the most fully known examples of environmental control of hereditary characters. The main facts have recently been reviewed

by Heidelberger.⁶ More than 50 distinct antigenic types of *Pneumococcus* are known. The character of each of these types is normally inherited within a clone; but by appropriate procedures a number of types have been converted into others and probably all conceivable transformations of one into another are possible. The conversion is accomplished by reducing one type into a non-specific form in which the capsule with its specific antigens (polysaccharides) are lacking, and then growing the non-specific form in the presence of heat-killed cells of the type to which conversion is desired. Thereupon, the form lacking a capsule acquires one which contains the type of polysaccharide antigen characteristic of the heat-killed cells. The essential material required for successful conversion is not the polysaccharide itself, but some other component of the heat-killed cell containing it.

The *Pneumococcus* situation may be compared with the killer situation in *Paramecium*. In the latter, transfer of a cytoplasmic substance from a killer cell to a sensitive cell containing gene *K* will result in the continued production of this substance and the killer phenotype which depends on it. Possibly all or most strains of *Pneumococcus* contain the gene or genes (or gene-like materials) required to control the continued production of each of the 50 different polysaccharides, and, as in *Paramecium*, when an appropriate substance essential for the synthesis of the polysaccharide is added to a cell that lacked it, the "gene" will determine its continued production. As in *Paramecium*, the gene seems to be unable to initiate its production, but can continue it when the proper substance is provided to start the gene going. The fact that any strain produces only one of these 50 polysaccharides would lead one to suppose that the same gene is involved in all 50 cases and that there are more than 50 cytoplasmic materials which the gene can act upon. As will be brought out below in the section on mutations, the gene *K* in *Paramecium* also appears capable of controlling the production of either of at least two somewhat diverse cytoplasmic substances.

Another class of possibly similar cases is the class of hereditary bacterial adaptations.^{6,7} A cell which is at first unable to produce a certain substance acquires the power to do so after subjection to an appropriate environment. In some instances the environment seems to act merely by favoring the growth of mutants which arise quite independently of the selective environment. It would appear worth while to investigate also the possibility that the hereditary properties of a strain could be altered by supplying the strain for a limited time with a small amount of the substance, or by extracts of strains that can produce it. Results of this general type have indeed been reported. *Eberthella typhosa*⁸ acquires the ability, previously lacking, of synthesizing tryptophane after growth in a tryptophane medium. *Propionibacterium pentasaceum*⁹

similarly acquires the ability to synthesize vitamin B₁ after growth in its presence. Superficially, the parallel to *Paramecium* suggests a similar genetic interpretation.

3. *Mutations*.—The observations on *Paramecium* show that mutations, in the sense of hereditary changes of characters, may have physical bases not at present recognized. According to prevailing views, the physical basis of a mutation is either a change in the gene or in the number and arrangement of the chromosomes and their parts. The hereditary change from sensitive to killer, however, can occur without any change in the gene, merely by addition of the essential cytoplasmic substance to the cell with the killer gene. The reverse hereditary change from killer to sensitive can occur simply by the loss of this cytoplasmic substance. Still another class of hereditary changes has been observed in *Paramecium*: strong killers which act quickly on sensitive cells have changed to weak killers which act slowly. The data indicate that this change is due to a change in the cytoplasmic factor, not to a change in the gene. In addition to these three previously unrecognized kinds of physical basis for a mutation, mutations of the well-known type have also been observed. The killer gene itself may mutate to the sensitive allele. The mutated gene is unable to control the further production of the cytoplasmic material even when some of it is present.

In a system of determination and inheritance such as prevails in *Paramecium*, a number of relations concerning relative mutation rates might be predicted. Two of these will be briefly indicated. First, the rate of mutation in opposite directions should as a rule be very unequal. For example, mutations from killer to sensitive would be expected more frequently than in the reverse direction. This follows from the fact that mutations from killer to sensitive will occur either if the cytoplasmic factor is lost or if the gene mutates to a form that cannot control production of the cytoplasmic factor; whereas mutation from sensitive to killer, in those cases in which the sensitive gene is present, requires both mutation of the gene and *de novo* origin of the cytoplasmic factor. Thus mutation in one direction follows either of two events while mutation in the reverse direction under certain conditions requires both of two events. These two events need not occur simultaneously, but the genic change must precede the cytoplasmic one.

Second, the observed mutation rate could, under certain conditions, be dependent upon whether the gene was in homozygous or heterozygous condition. To illustrate, in organisms homozygous for the sensitive allele, mutation of this gene to the killer allele would ordinarily be observed only if the cytoplasmic factor also arose subsequent to the gene mutation; but in organisms heterozygous for these genes and containing the cytoplasmic factor, every mutation of *k* to *K* would be directly de-

tected at autogamy, for the cytoplasmic factor is already present and being maintained. The result of this situation is to give under certain conditions higher observed mutation rates in heterozygotes than in homozygotes. This would be expected only for the mutation of genes that do not control a cytoplasmic factor into alleles that do.

4. *Development*.¹⁰—The fact that the determiners of the phenotype may lie outside of the nucleus and still be under genic control, as shown in the case of the determination of the killer phenotype, appears to have particularly important bearings on the prime problem of development: the production of different characteristics in cells with the same genes. With the occurrence of such determiners in the cytoplasm and, at least at certain stages, not in the nucleus, all that is required to account for the production of different characters in different cells with the same genes is to have differential segregation of these cytoplasmic determiners at cell division, a condition which has long been known to occur for a number of visibly different cytoplasmic components. The fact that the genes are unable to control the production of the substances once they are removed from the cell will prevent the cells which lose the cytoplasmic factors from developing the same characters as those cells which retain them.

Further pursuit of the possible implications of the system of determination and inheritance in *P. aurelia* seems at present less urgent than attempts to see whether comparable phenomena occur in higher organisms. From the discussion above on the problem of cytoplasmic inheritance in higher plants, one might expect these to provide the most favorable material for such study.

¹ Contribution No. 325 from the Department of Zoölogy, Indiana University.

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¹⁰ I am indebted to Dr. Boris Ephrussi for suggesting that the results on *Paramecium* might have bearings on problems of development.

THE RELATION BETWEEN NUMBER OF NUCLEOLI AND
NUMBER OF CHROMOSOME SETS IN ANIMAL CELLS

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The relation between the number of nucleoli visible in telophase or interphase nuclei and the number of haploid chromosome sets which are present has been studied extensively in plants (cf. the recent review by Gates¹). DeMol² first noted that diploid hyacinths have a maximum of two nucleoli, triploid three, and hypotetraploid four. This relationship became understandable when it was shown conclusively by Heitz³ that, during telophase, the nucleoli are formed at specific points on specific chromosomes, usually at secondary constrictions which either mark off a satellite or are located some distance from the end of the chromosome. Since as a rule, each haploid set of chromosomes includes a single chromosome with a "nucleolar organizer," the normal diploid nucleus contains two nucleoli. The number of nucleoli may therefore be used as a criterion for the identification of polyploid individuals or races within a species and, with certain restrictions, for the determination of phylogenetic relationships between different plant species.

The relation of nucleolar number to polyploidy has received much less attention from zoölogists, largely because, until recently, there was little interest in the study of polyploidy in animals in its various aspects. This has led Gates⁴ to the conclusion that "it is still uncertain in how far the number of nucleoli in animals can be used as an index of the number of sets of chromosomes." However, what evidence there is available in the zoölogical literature clearly points to the existence of the same constant relationship as obtains in plants, at least in ordinary somatic cells. Under special physiological conditions, however, (e.g., in gland cells and in growing oöcytes), the number of nucleoli may be greatly increased, apparently without a corresponding increase in chromosome number. The pertinent information on nucleolar number and heteroploidy will be reviewed briefly in this paper, and new evidence will be added from a study of the nucleoli in an extensive heteroploid series of larvae of the axolotl, *Amblystoma mexicanum*, and of larvae of mixed tigrinum-mexicanum ancestry.

That each haploid set of chromosomes may be associated with a single nucleolus was first indicated by Conklin's classical observations on the fertilization and cleavage of eggs of the gastropod *Crepidula*.⁵ Each of the germ nuclei, before their union, contains a single nucleolus, while in the telophase of all the cleavage mitoses two nucleoli appear which may

fuse into a single one if the resting period is prolonged. The cleavage nuclei are clearly dual, and each half-nucleus, which is descended from one of the germ nuclei, contains a single nucleolus.

Direct evidence for a constant relationship between number of nucleoli and chromosome number was found by Parmenter⁶ in his work on parthenogenesis in frogs. Epithelial nuclei of diploid tadpoles of *Rana pipiens* contain two nucleoli in a large percentage of the cells, and one in the remainder; haploid parthenogenetic tadpoles show only one nucleolus in all nuclei. In 1933 Parmenter extended his observations to a triploid and a mixed diploid-triploid larva of *Rana pipiens*, and a mixed diploid-triploid-tetraploid larva of *Rana palustris*.⁷ The triploid nuclei contained three nucleoli, the tetraploid four. The same relationship obtained in haploid, diploid and triploid cells of parthenogenetic individuals of *Rana fusca*.⁸

Corresponding figures were published by Porter⁹ for haploid, diploid and tetraploid cells of androgenetic larvae of *Rana pipiens*. A very extensive series of polyploid parthenogenetic larvae of *Rana nigromaculata* was investigated by Kawamura¹⁰ who found that most of the nuclei in triploid larvae contained three nucleoli, in tetraploid larvae four and in hexaploid larvae five or six.

The number of nucleoli was also recorded in germ cells of polyploid silkworms by Kawaguchi.¹¹ Diploid, triploid and tetraploid spermatogonia showed two, three and four nucleoli, respectively. Diploid oögonia contained a single nucleolus, triploid and tetraploid oögonia two, i.e., the number of nucleoli corresponded to the number of Z-chromosomes present, indicating that in this species the Z-chromosome carries the nucleolar organizer.

Since the number of nucleoli, together with measurements of the nuclear size, may give a reliable estimate of the degree of polyploidy of non-dividing cells (cf. Berger¹²) it promises to be particularly helpful in the study of partially polyploid organs or tissues in otherwise diploid individuals. In the normal liver of the rat, tetraploid and octoploid cells are abundant and may outnumber the diploid (Beams and King,¹³ Sulkin¹⁴). In the mouse liver, the nuclei fall into three main size classes whose volumes are approximately as 1:2:4 (Jacobj¹⁵), indicating that they are diploid, tetraploid and octoploid, respectively. Bieseke, Poyner and Painter¹⁶ have shown that these nuclei contain a maximum of four, eight and sixteen nucleoli. A few very large nuclei, presumably 16-ploid, showed up to thirty-two nucleoli. Each chromosome set of the mouse apparently contains two nucleolar organizers, a fact which might suggest that this species is tetraploid. However, some species of plants, which are unquestionably diploid, also possess four nucleolar organizers.

The numerous scattered observations on polyploid tumor cells have

been reviewed in the same paper.¹⁶ In their own investigations of the nuclear phenomena in mouse cancers these authors found evidence that the increased size of the cancer nuclei is a result of endomitosis which may lead to the formation of larger chromosomes with two, four, eight times, etc., the normal number of chromatids ("polytene chromosomes"), often combined with an increase in the number of separate chromosomes to the tetraploid, octaploid and higher levels. The actual valence of the nucleus, i.e., the total number of chromatids contained in it, is indicated by the number of nucleoli, although there is a strong tendency for the nucleoli to fuse, particularly in nuclei with polytene chromosomes. However, careful measurements of the volume of the nucleoli may still reveal their compound nature.

In the frequently highly polyploid somatic cells of water striders, fusion of two or more nucleoli seems to be the rule.¹⁷ Diploid and tetraploid nuclei usually show one nucleolus, rarely two. In more highly polyploid nuclei, the number of nucleoli increases but remains far below the number of chromosome sets which may be accurately determined by counting the heterochromatic X-chromosomes.

Prolonged studies of spontaneous and induced polyploidy in several species of salamanders have not produced information on the behavior of the nucleoli until recently. In the three species which were studied first, *Triturus viridescens*, *T. pyrrhogaster* and *Eurycea bislineata*, the chromosome number of living larvae may be determined with ease in whole-mounts of amputated tailtips where the epidermis of the transparent fin offers excellent material for the study of mitotic figures.¹⁸ The interphase nuclei, however, do not show distinct nucleoli following fixation in Bouin's and staining with Harris' acid haemalum, the technique which was found to be most satisfactory for the rapid preparation of the tailtips for chromosome counts. Last year, the investigations were extended to larvae of the axolotl, *Amblystoma mexicanum*.¹⁹ In this species and in mexicanum-tigrinum hybrids, a considerable number of the tailtip preparations show the nucleoli clearly in some interphase nuclei while in other nuclei they are not easily distinguished from the numerous chromocenters; frequently also the structure of the nucleus is partly obscured by pigment granules in the cytoplasm.

Sufficient observations have been made to show a good agreement between the maximum number of nucleoli visible and the number of chromosome sets present, as determined by chromosome counts in the same tailtip. The series includes haploid, diploid, triploid, tetraploid and pentaploid larvae (table 1). Some of these represent spontaneous aberrations in chromosome number, but the majority developed from eggs that were refrigerated immediately after laying. Haploid nuclei always contain a single nucleolus (Figs. 1 and 2), diploid nuclei two in the great majority

of cells, while in the remaining cells the nuclei contain one whose size may reveal its origin through fusion of two separate nucleoli (Figs. 3 and 4). Triploid individuals show three nucleoli in most of the cells, two in the remainder (Fig. 5); tetraploid nuclei have four nucleoli in over one-half of the cells, and three (rarely two), in the others (Fig. 6). In the tailtip of a pentaploid larva five nucleoli were found in a majority of the cells, while most of the remaining nuclei contained four (Fig. 7).

The demonstration of this simple numerical relationship in epidermis cells of axolotl larvae is particularly interesting in view of the fact that Dearing²⁰ described the formation of the nucleoli during telophase at the secondary constriction of a particular pair of chromosomes in epithelial cells of larvae of *Amblystoma tigrinum*. These observations, together with those of Kaufmann²¹ on ganglion cells of *Drosophila larvae*, were the first

TABLE 1

CHROMOSOME NUMBER, NUMBER OF NUCLEOLI AND NUCLEAR SIZE IN EPIDERMIS CELLS OF TAILTIPS OF AXOLOTL LARVAE

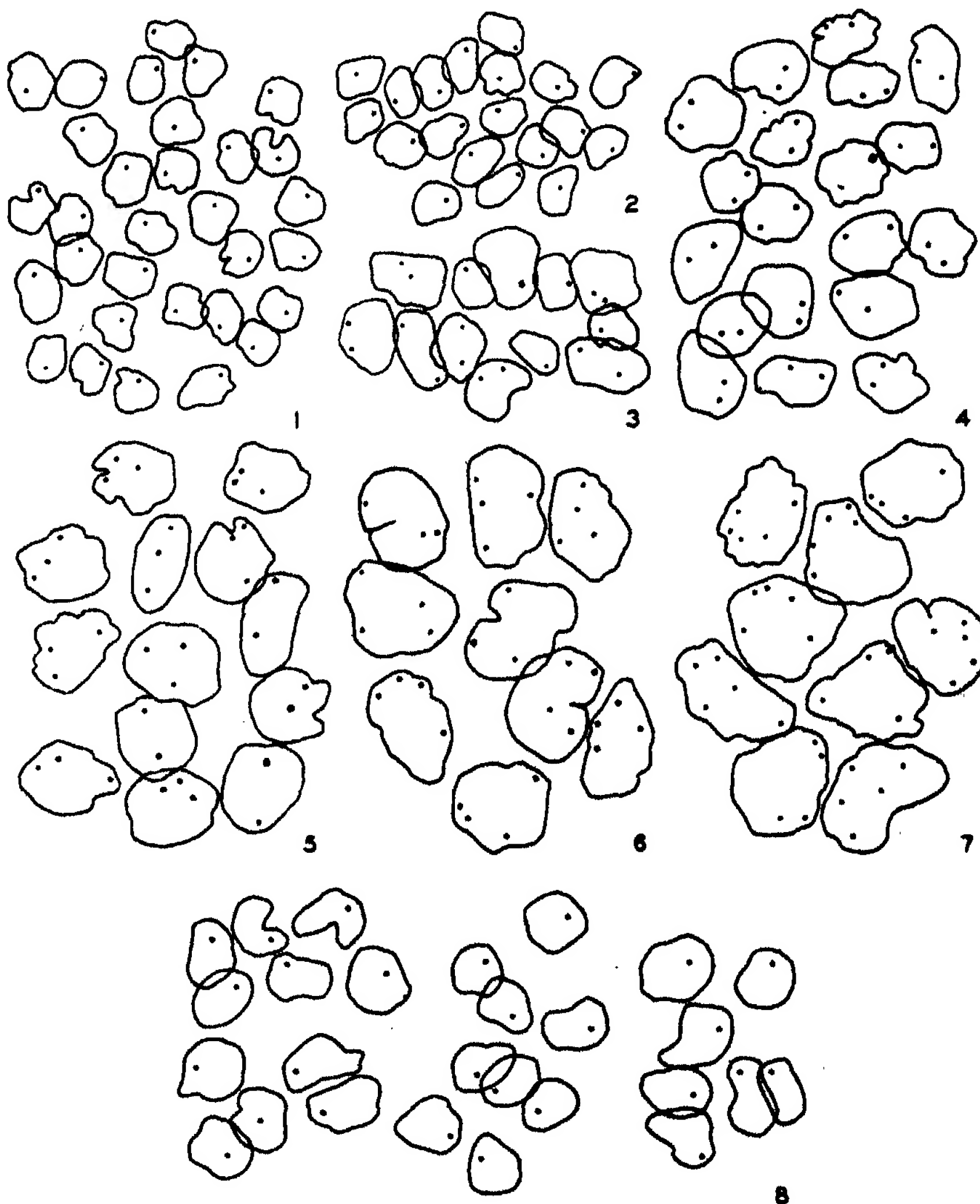
	HAPLOID	DIPLOID	TRIPLOID	TETRAPLOID	PENTAPLOID
Chromosome number	14	28	42	56	70
Maximum number of nucleoli	1	2	3	4	5
Area of 20 nuclei ($\times 630$) in square inches:					
No. of tailtips	14	100	96	2	2
Range	1.87-2.47	3.30-5.80	5.27-9.25	8.50-8.74	8.05-10.65
Average	2.23	4.37	6.67	8.62	9.35
Ratio	1.00	1.96	2.99	3.86	4.19

to show the existence of nucleolar organizers in animal cells. Dearing also found that the vast majority of epithelial cells contained two nucleoli, while the large single nucleolus visible in some cells was clearly a compound structure formed by the fusion of the two nucleoli during telophase. A single larva in his material, although unquestionably diploid, showed three nucleoli in each nucleus, the third nucleolus being associated with a chromosome that normally did not possess a nucleolus organizer.

Dearing's observations also offer a simple explanation for an apparent exception to the rule found among our axolotl larvae. The tailtip of one very abnormal larva showed epithelial nuclei within the diploid size range but with a single nucleolus (Fig. 8). In several mitotic figures from different regions of the tail fin the chromosome number was established as twenty-four, i.e., $2N - 4$. Obviously, one of the nucleolus-forming chromosomes was missing in this hypodiploid animal.

The availability of an additional criterion for the determination of the degree of polyploidy is particularly valuable in the axolotl in which accurate chromosome counts are often difficult to make because of the

scarcity of clear metaphase figures; as a result, identification of polyploid individuals was frequently based on measurements of nuclear size alone. As is seen from table 1, the area of camera lucida drawings of twenty



FIGURES 1-8

(For explanation, see opposite page)

epidermis nuclei is almost directly proportional to the chromosome number, except for the pentaploid nuclei in which the increase is relatively too small. However, only two pentaploid individuals were available; in

view of the considerable range of variation between individual tailtips it may be expected that measurements on more pentaploids will raise the average.

It should be pointed out that the epidermis nuclei of the tail fin are thin discs whose thickness increases very slightly with the chromosome number so that the area measured in the drawings is closely representative of the nuclear volume. This observation has a more general interest since it indicates that the thickness of the epithelium is kept more or less constant, regardless of the size of the individual cells which compose it. The cells are flattened to a greater or lesser extent to fit this condition. Preliminary observations on sections of various polyploid individuals have shown that a similar adjustment takes place in other thin layers of cells.

Summary.—(1) A review of the few reported observations on the number of nucleoli in nuclei of haploid and polyploid animal cells shows that the same relationship obtains as in plants, i.e., the nucleolar number increases in direct proportion to the chromosome number. In spermatogonia of polyploid silkworms and in epithelial cells of frog tadpoles the nuclei contain one nucleolus for each chromosome set. In normal liver cells and in cancer cells of the mouse the nuclei contain a maximum of two nucleoli for each chromosome set, although the great tendency of the nucleoli to fuse may obscure this relationship. In polyploid tissues of diploid water striders, fusion of the nucleoli occurs so generally that the nucleolar number is of little value in the estimation of chromosome numbers.

(2) New observations demonstrate a similar relationship in epithelial

EXPLANATION OF FIGURES

All figures are camera lucida drawings of epidermis nuclei from the tail fin of young axolotl larvae. The original magnification was $\times 630$; it was reduced to $\times 270$ in reproduction.

1. From a haploid larva ($N = 14$). All nuclei have a single nucleolus.
2. From the left (haploid) side of the tailtip of a haploid-diploid mosaic larva.
3. From the right (predominantly diploid) side of the same mosaic larva. A few haploid nuclei are present. The diploid nuclei have mostly two nucleoli; one nucleus contains a single large fusion nucleolus.
4. From a diploid larva. All nuclei but one with two nucleoli.
5. From a triploid larva. Of the thirteen nuclei shown, nine have three nucleoli, four have two.
6. From a tetraploid larva. Seven of the nine nuclei shown have four nucleoli, the other two have three.
7. From a pentaploid larva. Six of the nine nuclei shown have five nucleoli, three have four.
8. From a hypodiploid larva with twenty-four chromosomes ($2N-4$). Three groups of nuclei from different regions of the tail fin are drawn to show that all nuclei contain a single nucleolus; presumably, one member of the pair of chromosomes with nucleolar organizers is missing.

cells of heteroploid axolotl larvae, covering the whole range from haploidy to pentaploidy (Figs. 1 to 7, table 1). The maximum number of nucleoli visible in the nuclei of any individual corresponds to the number of haploid chromosome sets. A single larva with nuclei within the diploid size range showed a single nucleolus in all nuclei (Fig. 8). This animal was found to be hypodiploid ($2N - 4$); presumably, one of the missing chromosomes contained a nucleolus organizer.

(3) The available evidence supports the conclusion that the number of nucleoli may be used as a criterion in the diagnosis of completely polyploid individuals or of polyploid cells or tissues in animals as well as in plants.

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ON PRIMARY ATTRIBUTES OF ALLELES IN *DROSOPHILA MELANOGASTER*

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The mutant gene *cubitus interruptus* (*ci*), in the fourth chromosome of *Drosophila melanogaster*, causes a long gap in the fourth wing vein if it is present in hemizygous condition. If two, instead of one, alleles are present, *ci/ci*, the gap is shorter, and with three alleles, in the triplo-IV genotype, *ci/ci/ci*, often no gap appears² (Fig. 1). In contrast to this cumulative ef-

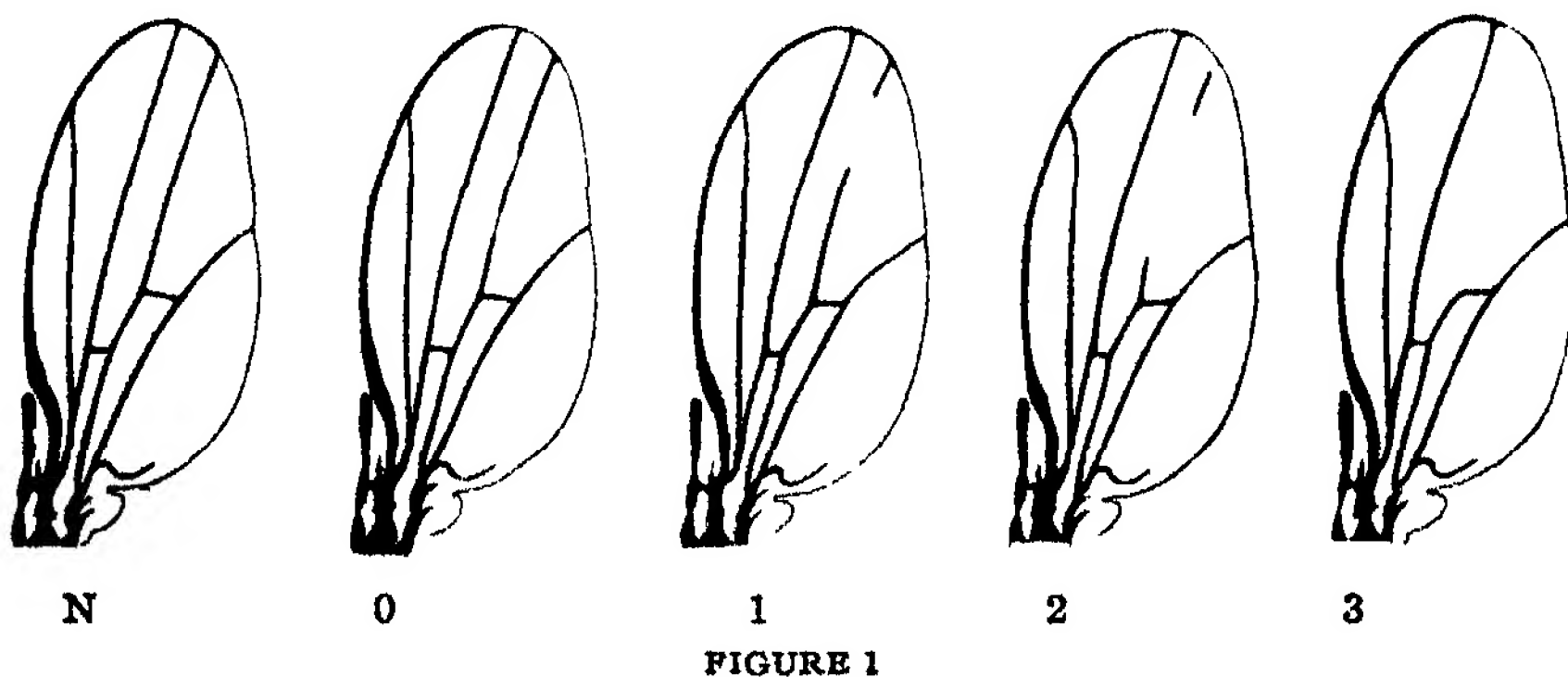


FIGURE 1
Classification of interruptions of cubital veins (for details see²).

fect of increased doses of *ci* is an antagonistic effect found when *ci* is combined with certain normal alleles of the *ci* locus.^{2, 3} Thus, the allele $+^3$ in hemizygous state produces, nearly always, normal venation, while flies with $+^3$ in combination with *ci*, $+^3/ci$, often have interrupted veins. To explain these and related results, the concept has been developed that a gene, in producing its ultimate phenotypic effect, acts at first upon a cellular substrate, *S*, and changes it into a product, *P*, which functions in the elaboration of a normal phenotype. It was further postulated that the action of a gene upon its substrate involves at least two factors: The "combining power," *c*, of the gene, as measured by the amount of *S* with which it is able to interact when *S* is in excess, and the "efficiency," *e*, with which the gene converts *S* into *P*. The operation of this system of primary gene action may be seen in its application to the apparently contradictory cumulative and antagonistic effects of *ci* on wing venation described above: In the former case it is assumed that *S* is present in excess of the combining power of the *ci* genes, but that one *ci* by itself is not able

to manufacture nearly enough P to give a normal wing. Doubling c by adding another ci or tripling by adding two ci brings the wing progressively closer to normal, as would be expected. The cumulative effect (toward normality) of additions of ci genes is thus due to the increased total amount of P produced, each ci gene combining with a fraction of S and contributing its increment of P .

The second case, in which ci , in combination with $+^3$, appears to decrease, rather than increase the approximation to the normal vein condition, can be explained if it is assumed that the efficiency of ci is less than that of $+^3$, and that the amount of S available is less than the maximum with which $+^3$ and ci together can combine. The alleles $+^3$ and ci are then competing for a limited S , so that they deprive each other of their full share of S . Since the efficiency of ci is less than that of $+^3$, ci does not utilize its share of S as effectively as $+^3$ would have—that is, it forms less P . Hence the total amount of P formed by both $+^3$ and ci working together is less than $+^3$ could have formed alone from S . This decreased amount of P results in a less normal wing.

The Relative Combining Powers of $+^3$ and ci .—While such an analysis permits a statement regarding the relative efficiencies e_{+^3} and e_{ci} of the two alleles concerned, it does not lead to deductions concerning the relative combining powers c_{+^3} and c_{ci} . Dosage experiments have shown that in diplo-IV flies the combining power of one ci allele is less than the available substrate, and that, in triplo-IV flies, the joint combining power of two ci alleles is less than the substrate available in such a constitution. Though it would greatly simplify the analysis if it could be assumed that the amount of substrate is alike in diplo-IV and triplo-IV flies, no relevant data are available, and the two constitutions must be treated separately. Furthermore, in neither case is it known how much of the substrate is claimed by a $+^3$ allele.

An estimate of the relative combining powers of $+^3$ and ci was made possible by an experiment in which the phenotype of triplo-IV flies of the constitution $+^3/ci/M-4$ was determined (table 14). It is seen that 56 out of 158 tested males with abnormal venation were of the constitution $+^3/ci/M-4$ (in addition to an unknown number among the normal overlaps not tested). Since nearly all $+^3/M-4$ flies are normal, and since the addition of an $M-4$ chromosome to various genotypes has been shown to result in phenotypic shift toward normality,² $+^3/M-4/M-4$ can be likewise regarded as normal. A comparison of the phenotypes of $+^3/ci/M-4$ (less than normal) and $+^3/M-4/M-4$ (normal) thus indicates that competition for a limited substrate occurs between $+^3$ and ci in $+^3/ci/M-4$. In other words, the joint combining power of the two different alleles c_{+^3} and c_{ci} is larger than S . It is known, on the other hand, that the phenotype of $ci/ci/M-4$ is less normal than that of $ci/ci/ci$ which means that the joint combining

powers of two *ci* alleles do not exhaust the substrate. In summary, $P(+^s/ci/M-4) < P(+^s/M-4/M-4)$, and therefore

$$c_{+^s} + c_{ci} > S, \quad (1)$$

and since $P(ci/ci/M-4) < P(ci/ci/ci)$, and therefore

$$c_{ci} + c_{ci} < S, \quad (2)$$

it follows, from (1) and (2), that

$$c_{+^s} > c_{ci}. \quad (3)$$

Relation (3) completes the description of the two alleles $+^s$ and *ci* in terms of both relative combining and efficiency factors. The normal allele possesses a greater combining ability and a greater efficiency than the mutant allele.

TABLE 1

(a) Not-Minute F_1 $\sigma^7 \sigma^7$ of $+^s/+^s \times ci\ ey^R/ci\ ey^R/M-4$,

N	PHENOTYPIC CLASSES*			
	0	1	2	3
134	53	100	14	..

(b) Of the above F_1 $\sigma^7 \sigma^7$ tested for being $+^s/ci\ ey^R/M-4$, by mating to unrelated females

0	1	2
47	99	12

(c) Of these the following were $+^s/ci\ ey^R/M-4$ as shown by appearance of at least three or in a few cases of two *M-4* F_1 flies

0	1	2
21	32	3

* *N* = uninterrupted distal section of 4th vein; 0 = uninterrupted but thinned section; 1, 2 = different progressive degrees of interruption; 3 = absence of whole section.

TABLE 2

	$\sigma^7 \sigma^7$					$\sigma^7 \sigma^7$				
	N	0	1	2	3	N	0	1	2	3
<i>ci</i> ^W / <i>ci</i> ^W	892	1	891
<i>ci</i> ^W / <i>ci ey</i> ^R *	4	980	6	959
<i>ci</i> ^W / <i>M-4</i> *	4†	3†	.	.	1	1

* These two groups of flies were derived from the same parents from crosses of *ci*^W/*ci*^W \times *ci ey*^R/*M-4*.

† Some of these may have been of Class 0.

The Relative Efficiencies of ci^W and other ci Alleles.—A similar analysis is possible for another mutant allele of the *ci* locus, *ci*^W. While *ci* in general acts as a recessive in combination with a wild allele (the allele $+^s$ being an exception to this statement) *ci*^W is dominant in heterozygous combination with any one of the three known normal alleles of the *ci* locus.³ Homozy-

gous ci^w/ci^w causes a very striking reduction of the venation, placing nearly all individuals into the most extreme Class 3 (table 2; all experiments referred to hereafter were made with stocks isogenic for all chromosomes except the fourth). Heterozygotes with normal alleles show wide variability with many individuals completely normal phenotypically (see table 5³). Heterozygotes of ci^w with the "recessive" ci are very similar to ci^w/ci^w homozygotes but seem to contain a slightly larger number of the less extreme Class 2 individuals (table 2). These facts permit certain deductions: Since, at 26°, the average phenotypes of heterozygotes of ci^w and any of the three normal alleles are more extreme than hemizygotes of any of the normal alleles, which practically always appear normal, it follows that

$$e_{ci^w} < e_{+c}, \text{ or } e_{+1}, \text{ or } e_{+3}. \quad (4)$$

Since it is further true that ci^w/ci is more extreme than $ci/M-4$, which has been shown to have its phenotypic mode in Class 2², it follows that

$$e_{ci^w} < e_{ci}. \quad (5)$$

This result can also be deduced from another experiment. In a cross of $ci^w/ci^w \times ci/ci/M-4$ the three genotypes ci^w/ci , $ci^w/ci/ci$, and $ci^w/ci/M-4$ were distributed among Classes 1 to 3 (table 3, a). Since it was shown that

TABLE 3

(a) Not-Minute F_1 ♂♂ of $ci^w/ci^w \times ci\ ey^R/ci\ ey^R/M-4$

<i>N</i>	0	1	2	3
.	.	3	78	671

(b) Of the above F_1 ♂♂ tested by crossing to unrelated females, for being $ci^w/ci\ ey^R/M-4$ (flies hatched during the first four days in four out of six cultures)

1	2	3
1	23	182

(c) Of these the following were $ci^w/ci\ ey^R/M-4^*$

1	2	3
.	1	75

* The appearance of at least three or in a few cases of two Minute flies was considered proof of the paternal constitution as $ci^w/ci\ ey^R/M-4$. The one P ♂ of Class 2 and two males in Class 3 gave rise to only one M fly in F_1 . These three M flies may not have been $M-4$ but newly arisen mutants.

only very few ci^w/ci flies belong to Class 2 and since progeny tests (table 3, b, c) prove that the same is true for $ci^w/ci/M-4$ flies, most of the individuals of Class 2 must have been of the constitution $ci^w/ci/ci$. Furthermore, it is known that the average phenotype of $M-4/ci/ci$ flies is considerably more normal than Class 2. Therefore, the "addition" of ci^w to the latter genotype, transforming it to $ci^w/ci/ci$, results in a decrease of normality, i.e.,

in competition for substrate between ci^w and ci with $e_{ci^w} < e_{ci}$. It may be added that this relation not only extends the result obtained in expression (4) but also confirms it *a fortiori* since it is known that

$$e_{ci} < e_{+^3}, \text{ or } e_{+^3}, \text{ or } e_{+^3}.$$

The Relative Combining Powers of ci^w and ci .—In order to obtain an estimate of the combining power of ci^w we employ reasoning similar to that used in comparing c_{ci} and c_{+^3} .

The phenotypes of flies of the constitution $ci/M-4/M-4$ were compared with those of the type $ci/ci^w/M-4$. The latter were found to be nearly exclusively of the most extreme Class 3 (table 3, *b*, *c*) while the former are known to be generally more normal. This means that the "addition" of ci^w to $ci/M-4/M-4$ results in a decrease in P , an indication of competition between ci and ci^w . Since, as stated before, it is known that two ci alleles do not exhaust the substrate available in a triplo-IV fly, whereas competition of ci and ci^w indicates limitation of substrate relative to the combining powers of these two alleles, it follows that

$$c_{ci^w} > c_{ci}. \quad (6)$$

The expressions (5) and (6) signify an interesting difference between the two mutant alleles

ci and ci^w : the combining power of ci^w is larger than that of ci , but its efficiency is smaller. No data are available at present which relate the combining power of ci^w to that of a normal allele. The relations of the three alleles $+^3$, ci and ci^w are illustrated in figure 2.

The Multiple Effects of ci^w and Its Primary Action (Table 4).—A very peculiar effect was observed when attempts were made to obtain flies hemizygous for ci^w . It was found that the genotype $ci^w/M-4$ is nearly fully lethal, with flies completing their pupal development but unable to emerge. Hemizygotes dissected from pupae invariably showed shortened, swollen,

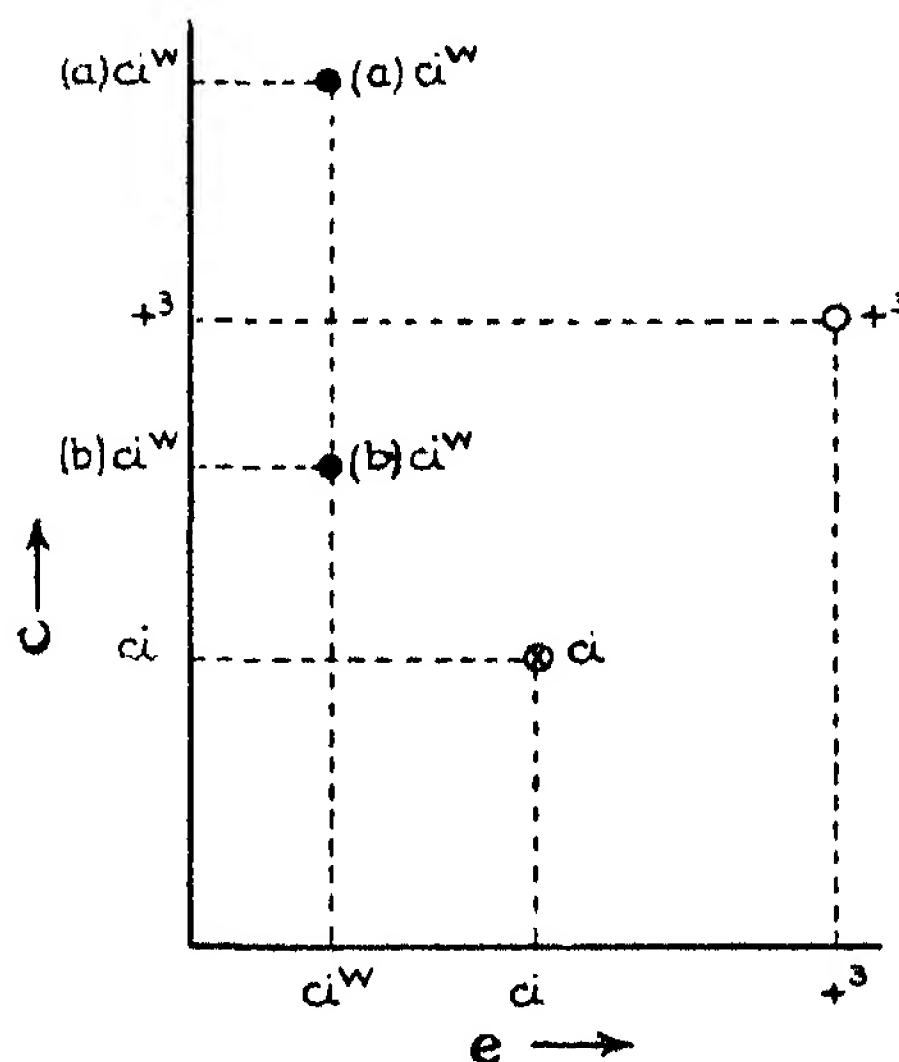


FIGURE 2

Diagram of possible relations of combining and efficiency factors of the alleles $+^3$, ci and ci^w . The seriation of the three e factors is established but not their relative distances. The seriation of the c factors is only established in so far as $c_{+^3} > c_{ci}$ and $c_{ci^w} > c_{ci}$; therefore two possibilities for c_{ci^w} are indicated: (a) $c_{ci^w} > c_{+^3}$, and (b) $c_{ci^w} < c_{+^3}$.

crippled legs so that their inability to hatch is obviously due to a great exaggeration of the slight crippling of legs occasionally found in homozygous ci^w/ci^w . Another characteristic exaggerated in $ci^w/M-4$ flies is the presence of extra bristles on the thorax, a feature found only rarely in our isogenic

TABLE 4
THE MULTIPLE EFFECTS OF ci ALLELES (FURTHER DETAILS IN TEXT)

GENOTYPE	VENATION CLASS	LEGS	EXTRA BRISTLES
$ci^w/M-4$	Usually N	Crippled	Few
ci^w/ci^w	Usually 3	Very slightly crippled	Very rare
ci^w/ci	Usually 3	Normal	None
$ci^w/+$	Usually 1 or 2	Normal	None
ci/ci	Usually 1 or 2	Normal	None
$ci^D/+$	Usually 3	Normal	None
ci^D/ci	Usually 3	Usually normal	Many
ci^D/ci^w	3	Crippled	Very many

stock of ci^w/ci^w . A single dose of ci^w is thus less effective in making for normality of legs and bristles than two doses. In contrast to this finding is the condition of wing venation. While it was not possible to classify wings from mature flies dissected out of the pupae, this was done with the wings of nine flies, out of an estimated two thousand, which had succeeded in hatching more or less completely. Eight of these showed a venation more normal than that of Class 3 (the ninth fly was of Class 3 phenotype, but had normal legs and no extra bristles. It was probably not $ci^w/M-4$ but rather ci^w/ci^w and had a Minute appearance due to a new mutation.). Of the eight $ci^w/M-4$ individuals seven seemed to have normal wings though it could not be excluded with certainty that some were of the slightly abnormal Class 0. The eighth fly was of Class 2 (table 5).

TABLE 5
PHENOTYPES OF $ci^w/M-4$

	N^*	0	1	2
Mature flies	7	0	1	1
Pupal wings	17	5	5	5
Total		29	5	6

* Some of these may have been Class 0.

The possibility was considered that the eight individuals which had succeeded in hatching might have been a selected group with unusual normality of wing venation. Accordingly, the phenotype of early pupal stages—before any selective influences would presumably have entered in—was ascertained. As Waddington (1940⁵) has shown, the typical venation of ci is visible in individuals of less than two days' pupal age. We found that the crippled legs are also clearly differentiated from normal legs at this time so that dissections of pupae from the cross $ci^w/ci^w \times M-4/ci$ enabled

us to ascertain the constitution as $ci^w/M-4$ (crippled) or ci^w/ci (normal legs). Thirty-two wings, from 19 individuals of the genotype $ci^w/M-4$, were fixed and stained in Delafield's hematoxylin and mounted in Canada balsam (table 5). Twenty-two wings were either completely normal, or of Class 0. In ten cases, the wings belonged to Classes 1 and 2. The controls, ci^w/ci , were all of Class 3 (13 specimens). There is thus no doubt that $ci^w/M-4$ (one ci^w) are more normal in venation than their ci^w/ci^w sibs (two ci^w).

A still more remarkable fact is that the wing phenotype of $ci^w/M-4$ hemizygotes is even more normal than that of $ci^w/+$ heterozygotes which have their mode in Classes 1 or 2³ (see particularly the heterozygote $ci^w/+$ ³). How unusual this relation is will be realized most clearly when it is pointed out that both the $+^3$ and the ci^w hemizygotes make for largely normal wings but that the heterozygote between these two alleles is strongly abnormal. Except for some analogy with Hinton's (1942⁶) incompletely analyzed Ey^{Dark} allele in *Drosophila* the only comparable though not identical case known to us is that of the various sex alleles in *Habrobracon* where the hemizygotes are all males while the heterozygotes are females (Whiting, 1940⁷).

An attempt to incorporate these data into the concept of genic action referred to earlier has led to the following considerations:

On the basis of the dosage data from ci and its $+$ alleles it was postulated that the effect of all ci alleles in regard to venation is fundamentally identical, the variations from one allele to another consisting only of differences of the combining power with the substrate and of the efficiency in transforming the substrate. Under this assumption the effect of the venation reaction of different ci alleles may vary from zero, in case the c or e factor, or both, are zero, to various positive values. A negative value for the product of the gene-substrate interaction would be impossible. Just such a negative value, however, is demanded by the observation that two ci^w alleles cause less normal venation than one ci^w allele. Therefore, an additional hypothesis is required if this case is to conform with the postulate that all ci alleles act basically in an identical way on venation. Two possibilities suggest themselves: (1) It is conceivable that another locus, or other loci, besides ci , combine with the substrate S and transform it into a product (P) effective toward normal wing venation. (The existence of the "other" locus could be visualized as due to a "repeat" of the ci locus.) If S is limited, competition between this other locus and ci^w would result, and if the efficiency of ci^w is lower than that of the other locus, the overall effect toward normal venation would be less in the presence of two ci^w alleles than of one. (2) As an alternative explanation of the observed effect of the ci^w allele, it might be assumed that the ci^w allele interacts with the same substrate S in two different ways, one leading toward normality of venation, the other toward an unrelated product. Under this assumption it can be seen

that competition for S between the two reactions might occur in such a way that one ci^w allele obtains sufficient substrate for the venation reaction to lead to near-normality, while two ci^w alleles, or the two alleles $ci^w/+$ combined, are not successful in this respect. This hypothesis implies that each of the combining properties of the two reactions are not simply proportional to the number of ci^w alleles but approach saturation values with different velocities. If this were not so, the shares of the substrate which the wing and the other reaction obtain would remain the same in $ci^w/M-4$ and ci^w/ci^w .

The hypothesis of two reactions of the ci^w allele competing for the same substrate may be fitted specifically to the leg or the bristle effect of ci^w . If it is the reaction toward normal leg or bristle development which competes with that for wing development, the correlation of crippled legs or extra bristles with normal wing in ci^w and of normal legs or normal bristles with deficient wing in ci^w/ci^w becomes understandable. Viewed from this angle the low efficiency of ci^w in terms of wing development would be due to the competing leg or bristle reaction whose efficiency in terms of normal venation may be zero.

It might be questioned whether the leg, bristle and wing effects are expressions of one and the same allele ci^w or rather stem from other loci in the fourth chromosome. The latter alternative is improbable since all these effects presumably arose simultaneously by mutation. In addition, the bristle effect exists in other alleles of the ci locus. While neither ci/ci nor $ci^D/+$ (ci^D is a dominant allele, lethal homozygously) show extra bristles typically, the heterozygote ci^D/ci has very many (Dr. Gertrude Heidenthal, unpubl.; confirmed by the present authors). The legs of ci^D/ci are only slightly abnormal, if at all, the sexcombs are large, resembling those characteristic for some ci^w stocks. The compound ci^D/ci^w is similar to the $ci^w/M-4$ hemizygote in having extremely crippled legs, which leaves it unable to emerge from the pupa case. The number of extra bristles is still larger than in ci^D/ci flies. Wings from seven individuals could be classified; they belonged to the extreme Class 3.

Whichever of the two explanations proposed for the venation effect of different doses of ci^w may approximate the truth, a single primary action of the ci^w allele seems ruled out by the facts: In order to allow for the cumulative effects toward normality of leg and bristle phenotype of two versus one dose of ci^w , a substrate must be *in excess* of the combining power of one ci^w allele, while in order to cause the decreased effect on venation of two versus one dose of ci^w , *limitation* of substrate relative to the combining power of one ci^w allele must exist. The apparent paradox implies at least two primary reactions of ci^w in which the relations of substrate to combining power differ. This difference may be due (a) to two basically different reactions of ci^w , either with two different substrates, or, if with one sub-

strate, with different combining powers. Or (b) the difference between wing and leg reactions may be due to two exactly identical reactions of ci^w with the same kind of substrate, S , which, however, take place in different cells during development, S being in excess of the combining power where leg formation is involved but S limited where venation is influenced. This possibility points to the need of further precision in the definitions of unitary vs. multiple primary gene action as discussed by various authors. Unitary primary gene action has been conceived as a single event in development from which one or more further events may follow while multiple primary gene action was implicitly regarded as signifying more than one basically different gene activity. It is, however, apparent that the same gene activity exercised at different places or times in development may find sufficiently different surroundings to result in very different effects. This possibility then traces multiple gene effects to multiple primary events though these events are of similar type. Such genic action may be called *repetitive*, to differentiate it from unitary as well as from multiple primary gene action in the older sense. Repetitive gene action may be due to prolonged, continuous or to recurrent gene activity.

Arguments related to the problems of primary gene action have been based on two different types of facts. In one, cases of multiple phenotypic effects of alleles were regarded by some as due to multiple primary action. Those opposed to this reasoning have pointed out that apparently unrelated phenotypic effects could be shown in several cases to depend on a single peculiar embryological mechanism ("spurious pleiotropism") and have argued that the principle of unity of gene action "may be adopted unless and until the occurrence of genuine pleiotropism can be conclusively demonstrated" (Grüneberg⁸). This viewpoint has not gone unchallenged (e.g., Dobzhansky⁹). Thus multiple phenotypic effects cannot be admitted as decisive evidence in regard to the type of primary gene action. The other kind of facts bearing on the problem of primary gene action is the existence of multiple alleles which can be arranged in two or more non-parallel series according to their effect on two or more different phenotypic characteristics. For example, among the R alleles in *Zea mays*, R^{co} and r^{co} both cause colored plant parts, the former allele giving colored, the latter colorless aleurone, while the alleles r^{or} and R^{or} both cause colorless plant parts but differ in that the former has colored, the latter colorless aleurone.¹⁰ An interpretation of facts like these in terms of multiple primary gene action is suggestive, but it could also be considered that each of the different alleles causes basically different unitary primary reactions. While such an assumption may appear less likely than the opposite one based on the postulate of basically identical effects of alleles of one locus on a given character, it should be pointed out that the postulate must depend on specific evidence of the type provided by dosage experiments in which the effect of

only one kind of allele, in different amounts, is studied. Such evidence is lacking in most cases. It is available for the *ci* locus: The recognition of repetitive or multiple primary action of *ci^w* depends on different dosage effects of *ci^w* and other alleles of the *ci* locus on two different characters. An attempt to regard the multiple effects of *ci^w* as secondary ones, all depending on unitary primary gene action, would meet with the difficulty that the effect of different doses of the *ci* allele shows the whole range of wing phenotypes from the extreme Class 3 to normality without any leg or bristle effects. Their appearance in the case of *ci^w* seems due to primary properties which distinguish the alleles *ci^w* and *ci*. Whether the leg and bristle effects themselves are due to a single primary gene reaction or whether they again are due to separate repetitive or multiple primary reactions remains undecided. Regardless of the future of the specific hypotheses proposed, it appears that a two-dimensional concept of genic action, in the form of a single reaction with constant amount of substrate and a combining and an efficiency factor, while giving a satisfactory account of one of the multiple effects, is not sufficient to explain the observations concerning more than one effect. The experimental facts seem to demand the introduction of additional primary attributes of the gene, such as multiple or repetitive action.¹¹ The further problem as to whether any one of the primary reactions considered is really a single one or consists of a whole group of reactions is completely beyond reach at present.

Summary.—1. A comparison of the effects on wing venation of the normal allele $+^3$ and the mutant allele *ci* shows that the normal allele has both a greater combining power with the substrate and a greater efficiency than the mutant allele.

2. The combining ability of the mutant allele *ci^w* is larger than that of *ci*, while the efficiency of *ci^w* is smaller than that of *ci*.

3. Hemizygotes *ci^w/M-4* possess mostly normal wings, but heavily crippled legs and extra bristles. Homozygotes *ci^w/ci^w* have abnormal wings, and mostly normal legs and bristles. Heterozygotes *ci^w/ $+^3$* are similar to, though less extreme than, *ci^w/ci^w*.

4. The bristle and leg characters of *ci^w* are presumably due to the *ci^w* locus and not to independent loci, since the compound between two other alleles, *ci^D* and *ci*, also shows marked excess of bristles and slight leg effects. The heterozygote *ci^D/ci^w* is similar, in bristle and leg abnormalities to *ci^w/M-4*.

5. An explanation of the action of *ci^w* in terms of a single primary reaction of this allele seems ruled out by the facts. It appears that either multiple, different, primary reactions of *ci^w* are involved or that a single reaction is repeated at different places or times in development causing different effects ("repetitive-action").

¹ Supported in part by a grant from the Rockefeller Foundation.

² Stern, C., *Genetics*, **28**, 441-475 (1943).

³ Stern, C., and Schaeffer, E. W., *Proc. Nat. Acad. Sci.*, **29**, 361-367 (1943).

⁴ The chromosome carrying *ci* also contained the eyeless allele *ey^R*. While useful as a marker, it has been shown to have no influence on the venation type. Its presence is indicated in the tables but not in the text.

⁵ Waddington, C. H., *Jour. Genetics*, **41**, 75-139 (1940).

⁶ Hinton, T., *Amer. Natur.*, **76**, 219-222 (1942).

⁷ Whiting, P., *Jour. Morph.*, **66**, 323-355 (1940).

⁸ Grüneberg, H., *Proc. Roy. Soc. B*, **125**, 123-144 (1938).

⁹ Dobzhansky, Th., *Genetics*, **28**, 295-303 (1943).

¹⁰ Emerson, R. A., Beadle, G. W., and Fraser, A. C., *Cornell Univ. Agric. Exp. Sta. Memoir*, **180** (1935).

¹¹ The case of the scute-alleles, which Tsubina (*Biol. Zh. Mosc.*, **4**, 1004 (1935)) has shown to act cumulatively toward normality for some bristles and in the opposite direction for other bristles, may find its explanation in terms of repetitive gene action in regions of different amounts of substrate.

ON WILD-TYPE ISO-ALLELES IN *DROSOPHILA MELANOGASTER*

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In experiments on the fourth chromosome locus, cubitus interruptus (*ci*), of *Drosophila melanogaster*, three homozygous stocks were obtained, which were isogenic except for the fourth chromosomes which came from three different sources. At the usual culturing temperature of 25-26°, all three stocks show normal wing venation and thus all seem to contain "the" normal allele of the *ci* locus. Tests reported below showed that each stock contains a different normal allele. Different alleles indistinguishable except by special tests will be called iso-alleles.

Origin of the Three Wild Iso-Alleles.—The first iso-allele occurs in the "Canton-Special" wild stock established by Bridges.² This allele is designated as $+^c$. Another wild iso-allele of *ci*, designated $+^2$, is in a fourth

TABLE 1

HOMOZYGOTES, 26°C. (UNLESS STATED OTHERWISE)

♀ ♀ + ♂ ♂	$+^c/+^c$	$+^1 ey^2/+^2 ey^1$	$+^1/+^1$
N*	2786†	1958	2900

* Phenotypic classes: N = uninterrupted distal section of 4th vein; 0 = uninterrupted but thinned section; 1, 2 = different progressive degrees of interruption; 3 = absence of whole section.

† The majority of these flies was raised at 18°C.

chromosome carrying the eyeless mutant ey^2 . The third iso-allele, $+^3$, was derived from a heterozygous culture of unclear origin. (The wild-type alleles of ci would ordinarily be designated $+^{ci-1}$, $+^{ci-2}$, etc., but since only this locus is referred to in this paper, the simpler symbols $+^c$, $+^2$, $+^3$ are employed throughout.) Table 1 records the numbers of homozygotes of these three alleles inspected at 26° or 18° . All were normal.

Tests of Homozygotes at Low Temperature (Table 2).—It is known that

TABLE 2					
HOMOZYGOTES, 14°C.					
	♀ ♀			♂ ♂	
	N		0	N	0
+ ^c /+ ^c	435	♀ ♀ and ♂ ♂: all N			
+ ² ey ² /+ ² ey ²	455		..	510	..
+ ³ /+ ³	918		24	801	21

low temperature accentuates the phenotypic effect of ci , whether produced by mutant alleles or a hemizygous wild allele.² If the three iso-alleles, in homozygous state, are raised at $14^\circ \pm 1^\circ$ (with occasional greater fluctuation of temperature) two of them, $+^c$ and $+^2$, still remain exclusively within the normal phenotype, but the third, $+^3$, is shifted in expression so as to lead to a small number of individuals which are slightly ci type.

Tests of Hemizygotes (Table 3).—At 26° flies carrying any one of the iso-

TABLE 3											
HEMIZYGOTES											
	26°C.				14°C.						
	<i>N</i>	♀	♀	0	<i>N</i>	♂	♂	0			
+ ^c / <i>M</i> -4	130	.		126	1	297	3	2	232	12	5
+ ² <i>cy</i> ² / <i>M</i> -4	1008	.		1122	.	251	31	3	276	27	.
+ ³ / <i>M</i> -4	317	3		368	3	141	10	1	75	9	.

alleles and the fourth chromosome $M-4$, which is deficient for a section including the ci locus, show the normal phenotype nearly exclusively, though a few are of the slightly abnormal Class 0. At low temperature proportionately more flies show the thinning or interruption of the fourth vein typical of ci . Differences between the alleles, in distribution of phenotypes, can be seen in table 3. The hemizygotes for $+^2$ and $+^3$ have a larger proportion of ci phenotypes than those for $+^c$. No clear difference appears between $+^2/M-4$ and $+^3/M-4$ flies. Together, the two tests, low temperature with homozygosity, and low temperature with hemizygosity, separate the three iso-alleles, the former demonstrating that $+^3$ differs from both $+^c$ and $+^2$, and the latter that $+^c$ differs from both $+^2$ and $+^3$.

Tests of Heterozygotes with ci (Table 4).—Flies heterozygous for ci^3 raised

TABLE 4
HETEROZYGOTES WITH $ci\ ey^R$

	26°C.					14°C.				
	N	♀ 0	♀ 1	♀ 2	♂ 0	♂ 1	N	♀ 0	♀ 1	♀ 2
$+^C + ey/ci\ ey^R$	1081	960	...	621	21	3	...
$+^2 ey^R/ci\ ey^R$	1273	1148	...	1043	14
$+^3 + ey/ci\ ey^R$	384	114	52	...	206	122	170	223	123	7
								135	209	123

at 26° fall clearly into two groups: $+^C/ci$ and $+^2/ci$ are all normal, while $+^3/ci$ flies are distributed over the phenotypic range of Classes *N* to 2. At low temperature $+^3/ci$ flies are further shifted toward abnormal venation. In addition some of the heterozygotes for $+^C$ and $+^2$ exhibit *ci* venation. The data show a difference in the expression of these two iso-alleles, the heterozygote for $+^C$ being more extreme than that for $+^2$. Hence, at low temperature all three heterozygotes differ.

The strikingly large number of flies with *ci* venation in the heterozygotes $+^3/ci$ at 14° invites a comparison with homozygous *ci/ci*. Such a comparison with various, independent, but isogenic experiments reported earlier² demonstrates that *ci* homozygotes are more extreme than the heterozygotes. In the latter the largest class of both females and males is Class 0 while in the homozygotes the mode is at Class 1 or 2.

Tests of Heterozygotes with ci^W (Table 5).—A very clear differentiation of

TABLE 5
HETEROZYGOTES WITH ci^W 26°C.

	♀					♂				
	N	0	1	2	3	N	0	1	2	3
$+^C/ci^W$	311	183	546	40	1	260	224	619	28	...
$+^2 ey^R/ci^W + ey$	239	244	763	157	5	118	223	896	179	...
$+^3/ci^W$	16	22	405	841	268	18	31	487	783	166

all three iso-alleles is accomplished, at 26°, by combining them with the allele ci^W . The heterozygotes $+^C/ci^W$ are distributed significantly more toward the normal phenotype than those of the constitution $+^2/ci^W$. The $+^3/ci^W$ flies are still more abnormal than the $+^2$ heterozygotes.

Possible Further Differences between the Iso-alleles.—In preliminary work with a homozygous $+^3$ stock, not isogenic with the stocks used in the experiments reported above, it was noticed, especially at lower temperature, that many individuals showed abnormally broad wings, sometimes with extra venation between the first and second veins. Occasionally a wing had an extra lobe on its anterior margin. These same abnormalities occurred after the stock had been made isogenic for the first, second and third chromosomes with the $+^C$ and $+^2$ stocks. Moreover, in the F_1 of the "isogenic" cross $+^3/+^3 \times ci/M-4$ none of the $+^3/ci$ individuals had the wing abnormalities, while their $+^3/M-4$ sibs were affected. This shows that the peculiar wing abnormalities are due to a recessive, fourth chromosome con-

dition located in that section around the *ci* locus which is deficient in the *M-4* chromosome. It is likely that the wing characteristics depend on the $+^s$ allele itself and constitute another peculiarity of this allele distinguishing it from $+^c$ and $+^s$. Besides the wing abnormality $+^s/M-4$ hemizygotes have enlarged sexcombs, a phenotype not found in the hemizygotes for the two other iso-alleles but which is one of the effects of the mutant allele *ci*^w. The sexcomb abnormality seems to differentiate further $+^s$ from $+^c$ and $+^s$.

Discussion.—The recognition of three distinct iso-alleles was accomplished in two ways: By testing them (1) in different environments, namely high and low temperatures, and (2) either as hemizygotes, or in heterozygous combinations with mutant alleles. A third kind of test, varying the genetic background, was not used, but might be expected to serve as another tool for the separation of iso-alleles.

To be accurate, the experiments reported do not establish beyond doubt allelic differences at the *ci* locus. They only narrow down the location of the genic differences demonstrated, to the *ci* locus and its immediate surroundings as delimited by the extent of the *M-4* deficiency. If a locus, or loci, outside the *ci* locus were responsible for the phenotypic differences observed, they would have to be dominant since the tests with heterozygotes rule out recessivity. Thus, an assumption of dominant modifiers of *ci*, located very near to the *ci* locus, is an alternative to regarding the *ci* locus itself as the cause of the differences. At present no means exist to prove this responsibility of the *ci* locus itself, but it seems the most likely assumption to make.

The discovery of iso-alleles is not new. Facts similar to those described here are known for the *vg* and *ct* loci in *D. melanogaster* (Mohr, 1932,⁴ Goldschmidt, 1937⁵). Another well-known example is that of two wild-type alleles of the *w* locus which were first recognized by their different somatic mutation rates after x-radiation (Timofeeff-Ressovsky, 1932⁶) and were then shown to give phenotypic differences in triploids in which they were tested with two doses of the mutant white allele (Muller, 1935⁷). The most striking case of numerous iso-alleles so far described is that concerning the bobbed locus in populations of *Drosophila hydei* (Spencer, 1938⁸). Other examples are the "specific modifiers," defined as genes which produce recognizable effects only in the presence of some main gene; they are but iso-alleles of their non-modifying alleles. In this group belong also alleles in some individuals of *Crepis* (Hollingshead, 1930⁹), *Godetia* (Hiorth, 1942¹⁰) and other organisms which act as lethals in species or racial crosses, while another allele of the locus concerned does not have lethal action. Within their own species or race, no differences have been observed between individuals homozygous for either the (potentially) lethal or non-lethal iso-allele. Still other types of iso-alleles are the multiple sex-alleles of *Ly-*

mantria (Goldschmidt, 1934¹¹) and *Habrobracon* (Whiting, 1943¹²). The peculiarity of the case described in the present paper is not so much the *existence* of the allelic differences but the fact that all three wild-type chromosomes tested were different. This seems to indicate, as did Spencer's findings, that the phenomenon of wild-type iso-allelism may be very frequent. It is probable that iso-allelism responsible for mutant phenotypes likewise is to be encountered. The "gene" then seems variable in many more ways than those responsible for striking effects.

It is possible to analyze somewhat further the differences between the wild iso-alleles of *ci* if the concepts of primary genic action, elaborated earlier,^{2, 13} are applied. According to these the action of an allele at the *ci* locus, in regard to wing development, depends (1) on the degree of ability, *c*, of the allele to interact with a cellular substrate, *S*, and (2) on an "efficiency factor," *e*, which measures the effectiveness of interaction in forming a product, *P*, which functions in the elaboration of the normal phenotype. On the basis of experimental facts, it is postulated that the action of different alleles depends on differences in *c* and *e*. As long as *S* sufficiently exceeds the combining power, *c*, of an allele, *P* is considered proportional to the product *c*·*e* of any allele.

(a) Since at 14° the hemizygotes of all three alleles $+^c$, $+^s$ and $+^l$ are less normal than the homozygotes, i.e.,

$$P(+^c, \text{ or } +^s, \text{ or } +^l/M-4) < P(+^c/+^c, \text{ or } +^s/+^s, \text{ or } +^l/+^l),$$

it follows that *S* exceeds the combining power of each allele,

$$c_{+^c}, \text{ or } c_{+^s}, \text{ or } c_{+^l} < S. \quad (1)$$

Since, however, it is known¹³ that the sum of the combining powers of $+^s$ and ci is greater than *S*, and that $c_{+^l} > c_{ci}$, it follows, that in the homozygote $+^s/+^s$

$$2c_{+^s} > S. \quad (2)$$

Hence, the abnormal phenotype at 14° of some $+^s/+^s$ individuals is not due to lack of interaction with the substrate, but to its inefficient utilization.

(b) Since the homozygote of $+^s$ is less normal than that for $+^c$ and $+^l$ it follows that

$$c_{+^s} \cdot e_{+^s} < c_{+^c} \cdot e_{+^c} \text{ or } c_{+^l} \cdot e_{+^l}. \quad (3)$$

Since the hemizygotes for $+^s$ and $+^l$, at 14°, are less normal than the $+^c$ hemizygote, i.e.,

$$P(+^s, \text{ or } +^l/M-4) < P(+^c/M-4),$$

it follows that

$$c_{+^s} \cdot e_{+^s} \text{ or } c_{+^l} \cdot e_{+^l} < c_{+^c} \cdot e_{+^c}. \quad (4)$$

Jointly, it follows from (3) and (4), that

$$c_{+1} \cdot e_{+1} < c_{+1} \cdot e_{+1} < c_{+c} \cdot e_{+c}. \quad (5)$$

(c) Finally, at 14°, the heterozygote $+^2/ci$ is more normal than the hemizygote $+^2/M-4$ whereas conversely the heterozygote $+^3/ci$ is less normal than the hemizygote $+^3/M-4$. Since it is known that $e_{ci} < e_{+1}$ or e_{+1} , the facts cited indicate that the "addition" of ci to $+^2$ does not result in sufficient competition for S to lead to a decrease of P as compared with $+^2/M-4$, while the "addition" of ci to $+^3$ deprives the latter of so much S , and ci interacts with S with so low an efficiency, that the total amount of P is less in the heterozygote $+^3/ci$ than in $+^3/M-4$. This means that

$$c_{+1} < c_{+3} \quad (6)$$

and, in view of (5), that

$$e_{+1} > e_{+3}. \quad (7)$$

No data are available to place the third wild iso-allele, $+^c$, in its proper order according to its c and e attributes.¹⁴

The general conclusion is that the wild iso-alleles may vary from each other in both their combining powers and efficiencies as had been shown for mutant alleles.¹³ These facts have wider significance! If two iso-alleles, x and y , differ in their c and e properties, it is to be expected that the difference in the amounts of S utilized by the two alleles (c_x vs. c_y), and the differences in the result of the utilization (e_x vs. e_y) would involve changes in other intracellular reactions. Thus, new points of attack for selective forces would originate. In general, selection should tend to bring the amount of substrate available to an allele to a safe minimum relative to its combining and efficiency powers. The amount of this substrate itself will depend on other genes, some of which may contribute to its elaboration and others which may be involved in its utilization. Any selection for amounts of substrate best fitted to various iso-alleles in one locus will result in the selection of specific iso-alleles of other loci. Hence, there will arise many different genotypes, phenotypically alike, which are due to different systems of iso-alleles. It can hardly be estimated how much of such concealed evolution, due to the kind of "germinal selection" described, is taking place at any time.

Summary.—1. Three stocks, isogenic except for the fourth chromosome, which came from three different sources, are all wild type at 25°–26°. If the three stocks are tested at low temperature, as hemizygotes, or in heterozygous combination with the alleles ci and ci^w of the *cubitus interruptus* locus, they are recognized as each carrying a distinct wild-type allele of ci (unless the alternative assumption is made of dominant modifiers of ci located very close to the locus of ci).

2. The existence of iso-alleles, defined as alleles indistinguishable except by special tests, is probably a general phenomenon.

3. The wild-type iso-alleles of *ci* are shown to differ from each other in their ability, *c*, to react with a cellular substrate, *S*, and in their efficiency, *e*, in transforming *S* into a product, *P*, which functions in the elaboration of normal venation. The iso-allele $+^2$ has a smaller *c*, but a larger *e* than the allele $+^3$.

4. It may be expected that natural selection leads to many different systems of iso-alleles in various loci.

¹ Supported in part by a grant from the Rockefeller Foundation.

² Stern, C., *Genetics*, 28, 441-475 (1943).

³ The chromosome carrying *ci* also contained *ey*^R. Similarly the chromosome carrying $+^3$ contained *ey*^s. The *ey*^R allele is without influence on the expression of *ci*. Except in the tables, references to the *ey* alleles will be omitted.

⁴ Mohr, O. L., *Proc. 6th Int. Congr. Genet. (Ithaca)*, 1, 190-212 (1932).

⁵ Goldschmidt, R., *Univ. Calif. Pub. Zool.*, 41, 285-296 (1937).

⁶ Timofeeff-Ressovsky, N. W., *Biol. Zbl.*, 52, 468-476 (1932).

⁷ Muller, H. J., *Jour. Genetics*, 30, 407-414 (1935).

⁸ Spencer, W. P., *Genetics*, 23, 170 (1938).

⁹ Hollingshead, L., *Ibid.*, 15, 114-140 (1930).

¹⁰ Hiorth, G., *Zeit. Vererbgs.*, 80, 289-349 (1942).

¹¹ Goldschmidt, R., *Bibliogr. Genetica*, 11, 1-185 (1934).

¹² Whiting, P. W., *Genetics*, 28, 365-382 (1943).

¹³ Stern, C., and Schaeffer, E. W., *Proc. Nat. Acad. Sci.*, 29, 351-361 (1943).

¹⁴ It might appear as if the data of tables 3 and 4 relative to $+^0/M-4$ and $+^0/ci$, at 14°, could be analyzed similarly to those relating to $+^2$ and $+^3$. However, as discussed earlier² the possibility exists that due to secondary difficulties the observed frequency of *M-4* types with *ci* venation may be too low as compared to that of non-*M-4* types so that conclusions should not be drawn from a comparison of the two similar phenotypes. In case of $+^2$ where the observed frequency of abnormal venation is higher in hemizygotes than in heterozygotes any error attributable to misclassification would only tend to decrease but not to simulate an actual difference, and in $+^3$ the differences in distribution are so large as to be beyond doubt.

CHROMOSOME COMPLEMENTS OF SOME SOUTH-BRAZILIAN SPECIES OF *DROSOPHILIA*

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For the last several months the writers have been engaged in making a preliminary survey of species of *Drosophila* which inhabit the states of São Paulo and Rio de Janeiro in Brazil. Among the rather numerous species examined, at least two proved to possess chromosomal complements of unusual interest. The purpose of the present note is to put on record an account of the chromosomes in these two as well as in some related species.

We have collected at Bertioga (state of São Paulo) and at Jacarepagua (Federal District) some females of a species apparently identical with *Drosophila prosaltans* Duda.¹ This species breeds well in the laboratory, and strains have been established without difficulty. Acetic orcein smear preparations of nerve cells of larval ganglia and of larval salivary glands have been made. The nerve cell metaphases show two pairs of V-shaped and one pair of rod-like chromosomes (Fig. 1, A-E). The V-shaped chromosomes appear equal-armed, one pair being perhaps slightly larger than the other. The centromere constrictions are usually well pronounced; in some cells (Fig. 1, C, D) one arm of one of the V's shows a secondary constriction at about the middle of its length, while the other V may show a secondary constriction in one limb in a submedian position. The rod-like chromosomes are about as long as an arm of the V-shaped ones, and have distinctly subterminal centromeres (Fig. 1, A, B, E). Metaphase plates in female and male larvae are indistinguishable; this indicates that the X- and Y-chromosomes are of about the same size and shape. The salivary gland cells show five long chromosome strands radiating from a well-developed chromocenter. In male larvae two of the five strands are paler than the remaining three. The paler strands represent the X-chromosome; the Y-chromosome is evidently heterochromatic, and the X-strands have no pairing mates in male cells.

Cells in which the chromocenter has been crushed by the pressure of the cover slip have been examined in order to determine the correspondence between the salivary and the metaphase chromosomes. In such cells the strands corresponding to a V-shaped chromosome frequently remain connected through the portion of the chromocenter formed by their heterochromatic regions. If a sufficient number of cells are examined this "vital artefact" gives data that are conclusive. In *D. prosaltans* most of the chromocentral mass goes with the two strands denoted in

figure 2 as *A* and *B*. These two strands are alike in female and male larvae; therefore, they represent a V-shaped autosome. In some larvae of the Bertioja cultures the *B*-strand showed two inversions separated by an uninverted section (see Fig. 2). Two other strands (*C* and X^2 in Fig. 2) remain connected through a small fragment of the chromocenter. Very surprisingly, one of these strands (X^2) proved to be much paler in male than in female larvae, while the other strand (*C*) is similar in either sex. This difference has been seen in many cells, and it is frequently quite

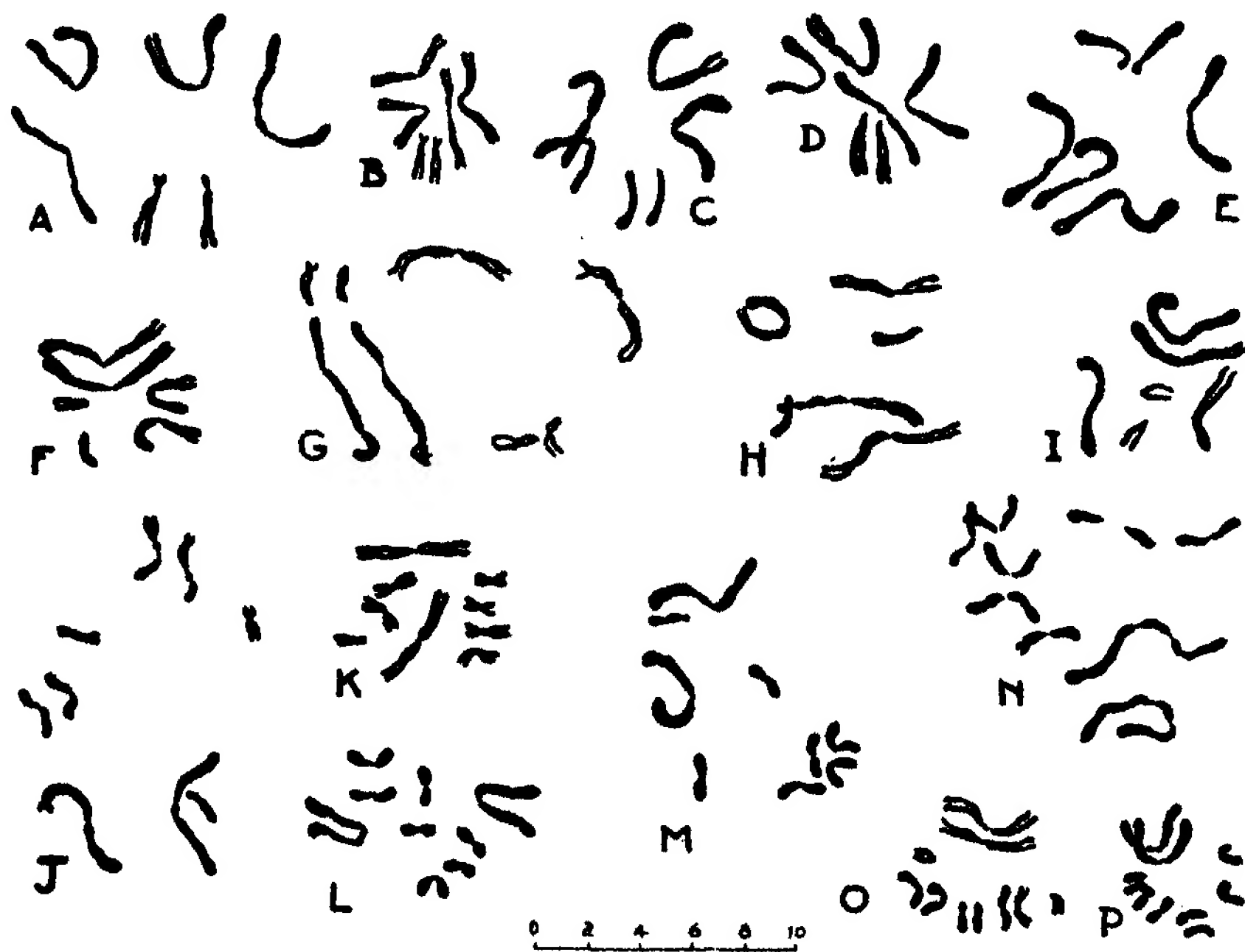


FIGURE 1

Nerve cell metaphases in *Drosophila prosaltans* (A-E), *D. sturtevantii* (F-I) and *D. annulimana* (J-P). The scale below represents 10 micra.

striking. One of the V-shaped metaphase chromosomes consists, therefore, of an autosomal and an *X*-chromosome limb. The fifth strand (X^1 in Fig. 2) is also paler in male than in female larvae; in cells with a fragmented chromocenter the X^1 strand lies frequently isolated from other chromosomes, the base having a small amount of heterochromatin. The X^1 strand corresponds to the rod-like chromosome of metaphase plates (Fig. 3).

We are forced to conclude that in *D. prosaltans* there are two *X*- and two *Y*-chromosomes: one rod-like and free (X^1 - Y^1) and the other attached to an autosomal rod, forming with the latter a V-shaped complex (AX^2 -

AY^2). The formula of the female is, consequently, $X^1X^1AX^2AX^2$, and of the male $X^1Y^1AX^2AY^2$. Such a chromosomal structure (Fig. 3) is unique in *Drosophila*; it must lead to a number of genetic complications. If, during the spermatogenesis, the X^1-Y^1 and the AX^2-AY^2 bivalents disjoin independently, four classes of spermatozoa, X^1AX^2 , Y^1AY^2 , X^1AY^2 , Y^1AX^2 , must be formed. The two last classes would give rise to inviable zygotes.

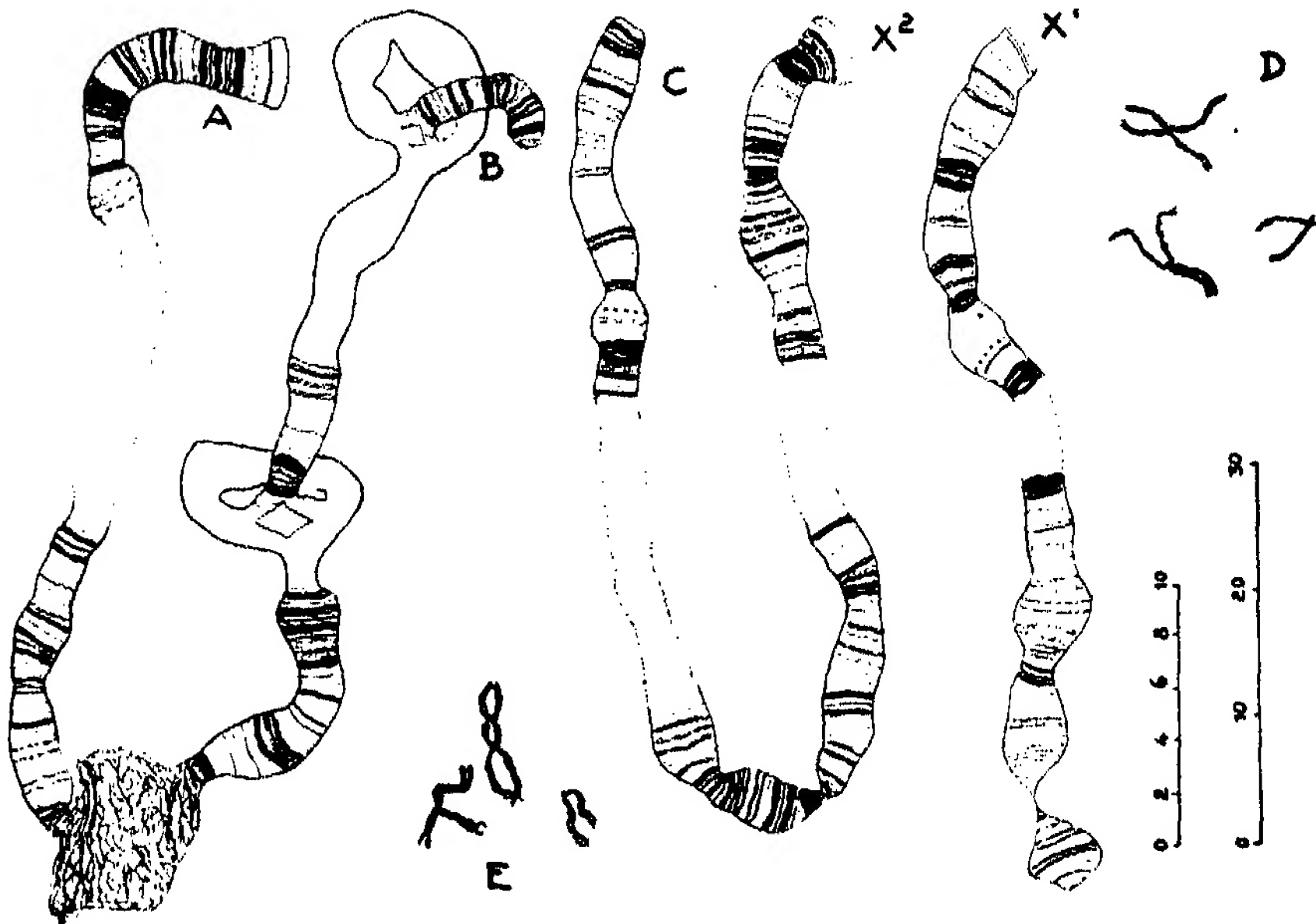


FIGURE 2

Salivary gland chromosomes (A, B, C, X^1 and X^2) and diakinesis configurations (D and E) in *Drosophila prosaltans*. The disc patterns are represented only in the basal and the terminal portions of the salivary gland chromosomes; the relative lengths of these chromosomes are not reflected in the drawings. The 30 micra scale applies to figures A, B, C, X^1 and X^2 , the 10 micra scale to D and E.

A somewhat analogous situation is observed in *D. miranda*, where the male is X^1YX^2 , but where only two types of sperms are produced; the X^1 and X^2 always go to the same pole, and the Y -chromosome goes to the opposite pole.² The Y -chromosome of *D. miranda* contains, however, some material homologous to that contained in the X^1 as well as to that in X^2 .³ How many classes of spermatozoa are formed in *D. prosaltans* is unknown. However, a species producing 50% of inviable eggs could hardly survive in nature; it is extremely likely that X^1AY^2 and Y^1AX^2 spermatozoa are either not formed or are not functional. Unfortunately, *D. prosaltans*, like most other species of *Drosophila* having highly spiralized testes, is

unfavorable for examination of spermatogenesis. A few smear preparations of the testes of young males have been made, but in none of them have good meta- and anaphases of the first meiotic division been encountered. Some cells showed stages of early diakinesis; three separate bivalents may be distinguished (Fig. 2, *D*, *E*). One of the bivalents consists of two rod-like chromosomes, invariably associated at one end only. The bivalents formed by the V-shaped chromosomes show a variety of structures, in some cases suggesting chiasmata, although this may be only a false appearance. The problem of chromosome disjunction in *D. prosaltans* is, evidently, an open one. Another open problem concerns the behavior of the genes carried in the autosomal pair associated with the X^2 - Y^2 chromosomes. The genes in this autosome should show all gradations between the normal autosomal inheritance and inheritance of sex-linked genes having alleles in the *Y*-chromosome (such as bobbed in *D.*

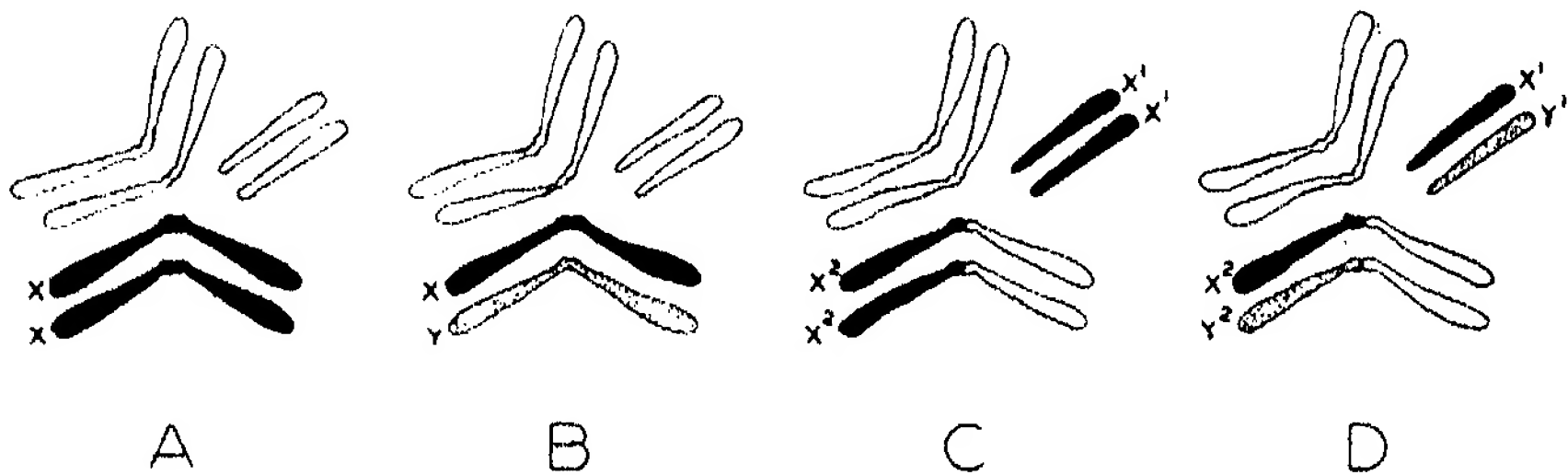


FIGURE 8

A scheme of the chromosome structure in *Drosophila sturtevantii* females (*A*) and males (*B*), and *D. prosaltans* females (*C*) and males (*D*). White—autosomes; black—*X*-chromosomes; stippled—*Y*-chromosomes.

melanogaster). The evolutionary fate of the genes located close to the centromere in the autosome associated with the *Y*-chromosome constitutes a still other interesting problem. Finally, a fourth problem which suggests itself is the origin of the unique heterochromosome mechanism of *D. prosaltans*. Fortunately, this problem can be somewhat clarified by comparison with a related species, namely, *D. sturtevantii* Duda.

We have laboratory strains of *D. sturtevantii*⁴ derived from females collected at Bertioga (state of São Paulo) and at Rio de Janeiro (the latter collected by Professor Hugo Souza Lopes). As in *D. prosaltans*, the nerve cell metaphases of *D. sturtevantii* show two pairs of V-shaped and a pair of rod-like chromosomes (Fig. 1, *F*-*I*). A close examination discloses, however, differences between these two species. In *D. sturtevantii* one of the V-shaped pairs is clearly longer than the other; in favorable cells it can be seen that one of the arms of the smaller V is appreciably longer

than the other. The rod-like chromosome is much shorter than either limb of at least the larger V; in contrast to *D. prosaltans*, the centromere constriction in the rod is not clearly subterminal. Very fortunately, the disc patterns in salivary gland chromosomes of *D. sturtevantii* proved to be sufficiently similar to those of *D. prosaltans*, so that the identification of

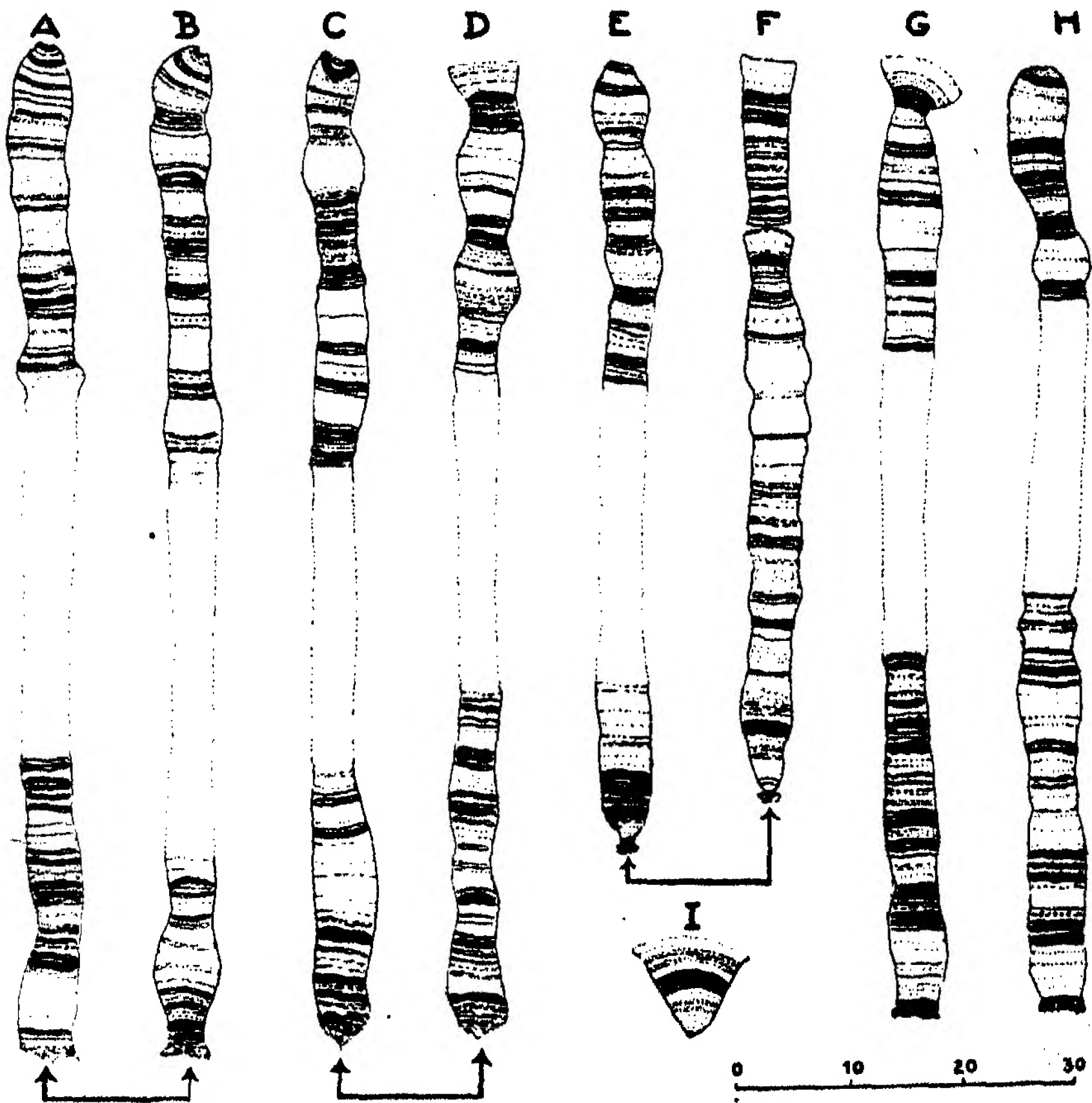


FIGURE 4

Salivary gland chromosomes of *Drusophila annulimana*. Except in the *F*- and *I*-chromosomes the disc patterns are shown only in the basal and the terminal portions; the relative lengths of the chromosomes are not reflected in the drawings. The scale below represents 30 micra.

the corresponding strands can be made without difficulty. In cells with a crushed chromocenter it can be seen that the *A*- and *B*-strands of *D. sturtevantii* are associated through a large chromocentral mass, forming, consequently, a V-shaped complex, like they do in *D. prosaltans*. In contrast to *D. prosaltans*, the *C*-strand of *D. sturtevantii* is frequently isolated

from the other strands, while the X^1 and X^2 strands are connected with each other through a small heterochromatic segment. As in *D. prosaltans*, the X^1 and X^2 strands in male cells of *D. sturtevantii* are paler than the other strands.

It is evident, therefore, that one of the V-shaped chromosomes of *D. sturtevantii* is an X-chromosome (or, in the male, an Y-chromosome), while the other V and the rod are autosomes (Fig. 3). The type of chromosome complement found in *D. sturtevantii* is "orthodox," and is, virtually certainly, ancestral to the aberrant type of *D. prosaltans*. The origin of the latter form from the former can be visualized only as taking place through reciprocal translocations between the X- and Y- chromosomes on one hand and an autosomal pair on the other (Fig. 3). V-shaped X- and Y- chromosomes exchange major portions of one of their limbs for most of a rod-like autosome (*sturtevantii*), giving rise to rod-like X- and Y- chromosomes and to a V-shaped pair consisting of autosomal and heterochromosomal limbs (*prosaltans*). This change must have involved some situations which, theoretically, might be discriminated against in natural populations. Indeed, the exchange of segments between the autosome and the X- and Y- chromosomes must have occurred in a species with *sturtevantii*-like chromosomes. At least for a time, translocation heterozygotes of a rather complex kind must have existed, for example, individuals with one V-shaped X, one rod-like X, one rod-like autosome and one V-shaped autosome-X-chromosome complex (and similar heterozygotes for the Y-chromosome-autosome translocation). With the disjunction mechanism now present in *D. prosaltans* as yet imperfect, the fertility of such translocation heterozygotes should have been below normal.

It is well known that, in *Drosophila*, individuals of a race, races of a species and species of the genus frequently differ in inversions of chromosome segments. A great majority of the inversions are paracentric (involving breaks on one side of a centromere), rather than pericentric (involving breaks on two sides of a centromere). The predominance of paracentric inversions in the phylogeny is understandable; paracentric inversion heterozygotes produce few, and pericentric inversion heterozygotes produce relatively many aneuploid gametes. Yet, it can be demonstrated that evolutionary changes sometimes take place through formation of pericentric inversions.⁵ In this respect, the chromosome complement of *D. annulimana* Duda is of interest. We possess some cultures of this very large species derived from females collected at Bombas, near Iporanga, state of São Paulo. The nerve cell metaphases in the females show (Fig. 1, *J-P*) ten chromosomes, including a pair of large V's, three pairs of much smaller V's and a pair of Y-shaped or rod-like bodies. The centromere constrictions are so strong that in some cells the two arms of

a V appear as two separate chromosomes; the long V and one of the short V's may show also very strong secondary constrictions in one limb (Fig. 1, *N*). Male cells show nine chromosomes: a pair of large and three pairs of small V's and an unpaired V or a rod (Fig. 1, *I-M*). *D. annulimana* is, consequently, *XX* in the female and *XO* in the male; this is the second known case of an *XO* species of *Drosophila*, the first being *D. orbospiracula* Patterson and Wheeler.⁶

The salivary gland cells of *D. annulimana* are very favorable for study. Eight relatively long and one very short chromosome strands can be counted without much difficulty. The disc patterns in the distal and the proximal portions of each strand are shown in Fig. 4, the middle portions being omitted except in shorter strands. The relatively long *A*- and *B*-strands are frequently associated in their proximal parts; they very likely represent the large V-shaped pair of metaphase chromosomes. The *C*-strand is almost always associated with *D*, and *E* with *F*; they probably correspond to two of the smaller V-shaped pairs of the metaphase plates. The *G*-strand is the *X*-chromosomes; *H* and the very small *I* probably belong together, although this has not been established by direct observation.

A majority of species of *Drosophila* so far cytologically investigated have five long and one very short chromosomal strands in their salivary gland cells, corresponding to the five variously associated or free rods and one dot-like chromosome of metaphase plates. Five free rods and a dot is the most likely ancestral condition of the chromosomal apparatus in the genus *Drosophila*, and it occurs frequently in species of the *repleta* group,⁶ of which *D. annulimana* is an aberrant member.⁷ The minimum of changes which have to be assumed to derive the chromosome complement of *D. annulimana* from the ancestral complement are as follows: (1) junction of two rods to form the large V of *annulimana*; (2) pericentric inversions in two rods with subterminal centromeres; (3) translocation of enough material from one of the rods onto the dot to transform the latter into a small V.

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¹ Duda (*Arch. f. Naturgesch.*, 91, 164 (1925)) has described "*Drosophila prosaltans* new species or variety of *saltans* Sturtevant"; the description is based on a single museum specimen from Hohenau, Paraguay. Sturtevant (*Univ. Texas Publ.*, 4213, 39 (1942)) gave the name *Drosophila sellata* to a species from Guatemala and Mexico which is very close to *D. prosaltans* Duda. Sturtevant's own comment is: "The chief reason for not applying that name (*prosaltans*) to it (*sellata*) is the distribution, plus the fact that I have not seen Duda's material." Our flies fit the description of *D. prosaltans* and differ only slightly from that of *D. sellata*.

² Dobzhansky, Th., *Jour. Genetics*, 34, 135-151 (1937).

³ Mac Knight, R. H., *Genetics*, 24, 180-201 (1939).

⁴ Described by Duda (*Arch. f. Naturgesch.*, 91, 167 (1925)) from a single Museum specimen from Mapiri, Bolivia. A very close, possibly identical, species has been named *Drosophila biopaca* by Sturtevant (*Univ. Texas Publ.*, 4213, 37 (1942)).

⁵ Miller, D. D., *Genetics*, 24, 699-708 (1939).

⁶ Patterson, J. T., and Wheeler, M. R., *Univ. Texas Publ.*, 4213, 67-109 (1942).

⁷ *Drosophila annulimana* is described by Duda (*Arch. f. Naturgesch.*, 91, 117 (1925)) as a member of his subgenus *Paradrosophila*. As delimited by Duda, this subgenus is not a natural group; it includes most diverse forms having in common only a single character, namely, prescutellar bristles. *Paradrosophila annulimana* is much closer to species of the repleta group of the subgenus *Drosophila* than to such representatives of *Paradrosophila* as *bromeliae* Sturtevant. A new species close to *bromeliae* is found in São Paulo. It has three pairs of about equally long V-shaped chromosomes.

A TYPE OF UNIVERSAL ARITHMETICAL FORMS

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1. By a few obvious changes in the wording, all that follows may be restated for any unique factorization domain. The type of forms considered is defined in §§2, 3.

If the integer $n \neq 0$ is expressible in the form $x^a y^b \dots w^c$, where all the letters denote integers, a, b, \dots, c are constants and (without loss of generality) are all different, we shall say that n has the *index* $[a, b, \dots, c]$, and write

$$I(n) = [a, b, \dots, c].$$

The order of a, b, \dots, c within the bracket is immaterial.

The set of all integers having the index $[a, b, \dots, c]$ will be written $I^{-1}[a, b, \dots, c]$. Hence if at least one of a, b, \dots, c is 1, $I^{-1}[a, b, \dots, c]$ is $I^{-1}[1]$, the set of all integers except zero.

Let p_1, \dots, p_s be the distinct positive prime divisors of $n > 1$, and let

$$t = \prod_{i=1}^s p_i^{h_i}, \quad t = x^a y^b \dots w^c,$$

be the prime decomposition of t and a representation of t with index $[a, b, \dots, c]$ and $x, y, \dots, w > 0$. Denote by $L(t; a, b, \dots, c)$ the number of such representations. Then, on replacing x, y, \dots, w by their prime decompositions, it is evident that

$$L(t; a, b, \dots, c) = \prod_{i=1}^s L(t_i; a, b, \dots, c),$$

in which $(t_i; a, b, \dots, c)$ is the number of sets of solutions (x_i, y_i, \dots, w_i) of

$$ax_i + by_i + \dots + cw_i = t_i$$

in integers ≥ 0 . Thus $L(t; a, b, \dots, c)$ is defined for integers $n > 1$.

By definition, $L(1; a, b, \dots, c)$ is the number of solutions of $1 = x^a y^b \dots w^c$ in positive integers; $L(1; a, b, \dots, c) = 1$.

If $t < 1$, $L(t; a, b, \dots, c)$ is defined as the number of representations of t in the form $x^a y^b \dots w^c$ by integers x, y, \dots, w ; $L(t; 2a, 2b, \dots, 2c) = 0$. If $t < 1$ and a, b, \dots, c are not all even, let precisely g of them be odd. Then by changes of an odd number of signs of the variables having these g odd exponents,

$$L(t; a, b, \dots, c) = g_1^2 L(-t; a, b, \dots, c), \quad t < 1,$$

where g_1 is the greatest integer $\leq (g + 1)/2$.

If the number of variables x, y, \dots, w is h , it follows that

$$L(t; a, b, \dots, c) = O((t_1 \dots t_h)^{h-1}), \quad |t| > 1.$$

2. A form $F(x, y, \dots, w)$ in the variables x, y, \dots, w is defined here to be a polynomial in the variables with integer coefficients and no constant term. (In the case of any unique factorization domain the coefficients are in the domain.) The number of terms in a form will be called its *extent*.

If integer values x', y', \dots, w' , all different from zero, of x, y, \dots, w can be found such that $n = F(x', y', \dots, w') \neq 0$, we shall say that n is *represented* by (x', y', \dots, w') in $F(x, y, \dots, w)$. It is to be noted that representations of zero are undefined. (If zero values of the variables were admitted, the problem of representation for the type of forms considered here would reduce to the case discussed.)

If all integers in the set $I^{-1}[a, b, \dots, c]$ are represented in $F(x, y, \dots, w)$, we say that the form is *universal for the set*, write

$$F(x, y, \dots, w), \quad [a, b, \dots, c],$$

and call $[a, b, \dots, c]$ an *index* of $F(x, y, \dots, w)$. In particular,

$$F(x, y, \dots, w), \quad [1, b, \dots, c]$$

states that all integers $\neq 0$ are represented as defined in $F(x, y, \dots, w)$.

For all the forms considered a lower limit for the number $L(t)$ of representations of each integer t in the set $I^{-1}[a, b, \dots, c]$ for which $F(x, y, \dots, w)$ is universal is $L(t) \equiv L(t; a, b, \dots, c)$. In particular, if all integers $t \neq 0$ are represented, t has at least $L(t; 1, b, \dots, c)$ representations.

3. The general form of extent s in the variables x_1, x_2, \dots, x_n of the type considered here may be written

$$\sum_{i=1}^s d_i x_1^{a_i} x_2^{b_i} \dots x_n^{c_i},$$

where the d_i are integers $\neq 0$ and the exponents are integers subject to the conditions

$$a_i, b_i, \dots, c_i \geq 0, \quad i = 1, \dots, s;$$

$$\prod_{i=1}^s a_i = \prod_{i=1}^s b_i = \dots = \prod_{i=1}^s c_i = 0;$$

$$\sum_{i=1}^s a_i > 0, \quad \sum_{i=1}^s b_i > 0, \dots, \quad \sum_{i=1}^s c_i > 0.$$

4. Before indicating the method of proof we give some examples for forms of extent 3. Similar results for forms of any odd extent are readily obtainable by the method indicated; forms of even extent require other considerations, which apply also to forms of odd extent.

For a form representing all integers $t \neq 0$ an index is given so that the limit $L(t)$ may be written down. Those of these forms in which two signs are positive and one negative represent any $t > 0$ by at least $L(t)$ sets of values all > 0 of the variables. Any integer values which make the exponents ≥ 0 may be assigned to m, n ; any zeros in the resulting index symbol are to be suppressed.

$$x^{m+1}y^n + yz^{m+1}w^{n-1} - w^nxz^m, \quad [n-1, n, m+1, nm+n+1, nm+m+1]. \quad (1)$$

$$z(x^{m-1}y^{n+1} + w^{n+1}) - yx^mw^n, \quad [1, m+n, m+2n]. \quad (2)$$

$$xy^nz^{m-1} - z^mw^n + w^{n-1}yx^m, \quad [1, n, m, nm+m-1]. \quad (3)$$

$$x(y^{n+1} + z^{m+1}) - y^mz^n, \quad (4)$$

of which an index is the set of all the numbers common to $[n+1, n+2, \dots, 2n+1]$, $[m+1, m+2, \dots, 2m+1]$.

$$x^my^nz + z^2y^{n-1}w^m + w^nx^{m+1}, \quad [n, m+1, n+m+1]. \quad (5)$$

All of equations (1) to (3), and (5) are homogeneous. Another example of a nonhomogeneous universal with two arbitrary integer exponents is

$$x^ny^{m-1} + y^mz + z^2x, \quad [1, 3, 5, \dots, 2n+1]. \quad (6)$$

The representations in equations (7) and (8) contain integer parameters:

$$x^my^2z^{n-1} + z^nyw^m + w^{m+n}x, \quad [1, m+n, m+n+1], \quad (7)$$

has 1-parameter representations of all integers $t \neq 0$; every t^4 has at least $L(t; 1, 2, 3)$ 4-parameter representations in

$$x^4 + y^4 + xyzw. \quad (8)$$

The next four are typical of a class in which the sum of two terms can be written $x(y^n + w^n)$:

$$\begin{aligned} x(y^3 + w^3) + zyw, & \quad [1, 3, 4, 6]; \\ x(y^3 + w^3) + zy^2w^2, & \quad [1, 3, 4, 5]; \\ x(y^5 + w^5) + zyw, & \quad [1, 5, 6, 10, 15, 20]; \\ x(y^5 + w^5) + zy^4w^4, & \quad [1, 2, 6, 7, 8, 9, 10]. \end{aligned} \quad (9)$$

As examples of forms with index different from [1],

$$\begin{aligned} xy^3 - yz^3 + zx^3, & \quad [2]; \\ x^4y + y^2xz^2 + z^5, & \quad [3, 5, 12, 17]; \\ x^5 + zx^2y^2 + y^5, & \quad [2, 3, 5]; \\ x^4y^2 + yz^5 + z^3x^3, & \quad [5, 6, 11, 13]. \end{aligned} \quad (10)$$

5. A sufficient indication of a method for obtaining indices of forms of odd extent of any degree in any number of variables is given by two simple remarks: $I^{-1}[1]$ is the set of all integers; a solution of any one of a given set of equations $E_1 = 0, \dots, E_s = 0$ is a solution of $E_1 \dots E_s = 0$. (All of the examples in §4 refer to the case $s = 2$.) When at least one of the equations, say, $E_j = 0$, can be written $E_j' = E_j''$, where E_j', E_j'' are monomials in the variables and have no variable in common, the complete integer solution of $E_j = 0$ expresses each of the variables as a monomial in integer parameters. Applying this and the known methods for finding the solutions described to the second remark above, we find the indices. In all of the examples in §4 the coefficients in the forms are ± 1 . Any numerical coefficients may be introduced as in extending the solution of $E_j' = E_j''$ to that of $a_j E_j' = b_j E_j''$, where a_j, b_j are any given integers. If each of $E_1 = 0, \dots, E_s = 1, s > 1$ can be written in the same form as E_j , the extent of the form whose index is to be found is $2^s - 1$.

THE APOLLONIAN PACKING OF CIRCLES

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A theorem of the senior author concerning the covering of the plane by circles is established in *Scripta Mathematica* for March, 1943.¹ In this paper we give an analytic proof of the same theorem. The subject is also of interest in geology (packing of sand, porosity problems) and other fields of science.

1. *Definitions and Statement of the Theorem.* Let us effect a covering

of the plane with mutually external, equal circles each tangent to six others. We call this configuration the *circlex*. That part of the plane which is external to the circles of our covering consists of curvilinear triangles. In each of these we inscribe a circle, and continually keep inscribing circles in each curvilinear triangle obtained thereafter *ad infinitum*. We call the latter configuration an *Apollonian packing of circles in the plane* (or *hypercirclex*).

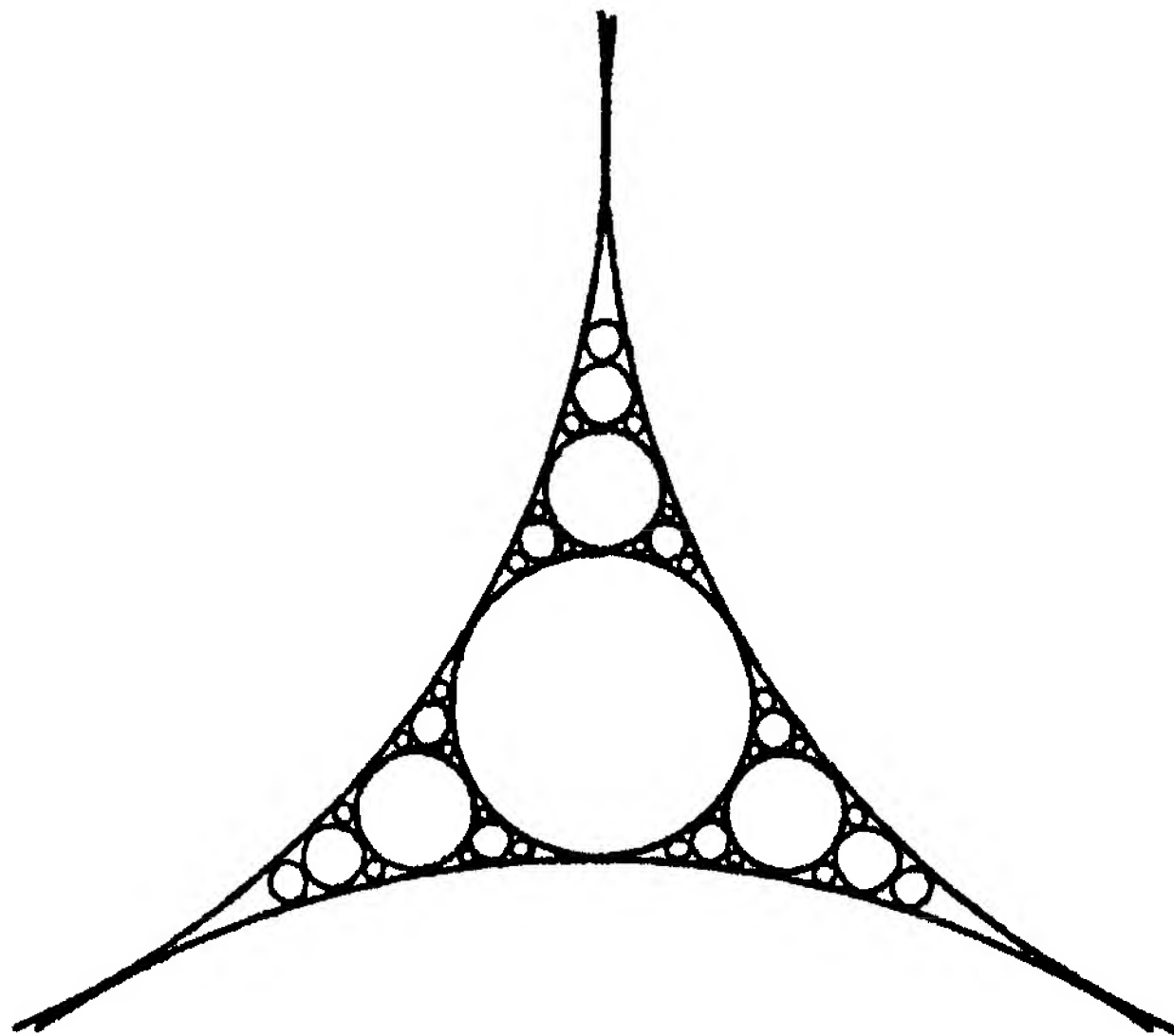


FIGURE 1

Let $T(a, b, c)$ denote the curvilinear triangle formed by the three mutually and externally tangent circles C_a, C_b, C_c with radii a, b, c , respectively. The term *Apollonian packing of circles in $T(a, b, c)$* is defined similarly, i.e., we inscribe a circle in $T(a, b, c)$ and continually keep inscribing circles in each resulting curvilinear triangle (see Fig. 1).

Let a set G of mutually external circles each lying in $T(a, b, c)$ be given. Then the term *vacancy of $T(a, b, c)$ relative to G* signifies the set of points in or on $T(a, b, c)$ which are not interior to an element of G .

We now state the

THEOREM: *The area of the vacancy of an Apollonian packing of circles in any curvilinear triangle is zero. In other words, the sum of the areas of the infinity of circles of an Apollonian packing in $T(a, b, c)$ is equal to the area of $T(a, b, c)$.*

We note that since our theorem is true, then because of the symmetrical properties of our initial covering of the plane, it is an immediate conse-

quence that the area of the vacancy of an Apollonian packing of circles in the plane is zero.

Our method of proof is different from that of the previous paper in the sense that we choose a different sequence of diminishing vacancies, it being easier to determine a bound for the ratio of diminution at each stage.

Let H denote the set of circles associated with an Apollonian packing in $T(a, b, c)$.

Let $C(x, y, z)$ denote the circle inscribed in $T(x, y, z)$.

The most natural method of exhausting the circles of H would be to group them into subdivisions as follows: We inscribe a circle in $T(a, b, c)$;

then we inscribe a circle in each of the three curvilinear triangles formed; then in the 3^2 triangles formed, etc. Thus, for $n = 1, 2, 3, \dots, i, \dots$

1st subdivision: $C(a, b, c)$

2nd subdivision: $C(C(a, b, c), b, c)$,
 $C(C(a, b, c), a, c)$, $C(C(a, b, c), a, b)$.
 If the circles of the $(n - 1)$ st subdivision are:

$C(a_1, b_1, c_1)$, $C(a_2, b_2, c_2)$, \dots ,
 $C(a_k, b_k, c_k)$

where $k = 3^n - 2$, then the circles of the n th subdivision are:

$C(C(a_j, b_j, c_j), b_j, c_j)$, $C(C(a_j, b_j, c_j), a_j, c_j)$, $C(C(a_j, b_j, c_j), a_j, b_j)$

where $j = 1, 2, \dots, 3^{n-2}$. But, the most natural procedure is not the most convenient in this case.

We define what we shall call a *necklace packing* (see Fig. 2) of a curvilinear triangle $T(a, b, c)$. It is the set of circles which comprises $C(a, b, c)$, the three circles inscribed in the curvilinear vertex triangles, the three circles inscribed in the new vertex triangles, and so on. Thus for $n = 1, 2, \dots, i, \dots$ it contains the circles belonging to all of the following subdivisions:

1st subdivision: $C(a, b, c)$

2nd subdivision: $C(C(a, b, c), b, c)$, $C(C(a, b, c), a, c)$, $C(C(a, b, c), a, b)$.

If the circles of the $(n - 1)$ st subdivision are:

$C(a_1, b, c)$, $C(a_2, a, c)$, $C(a_3, a, b)$

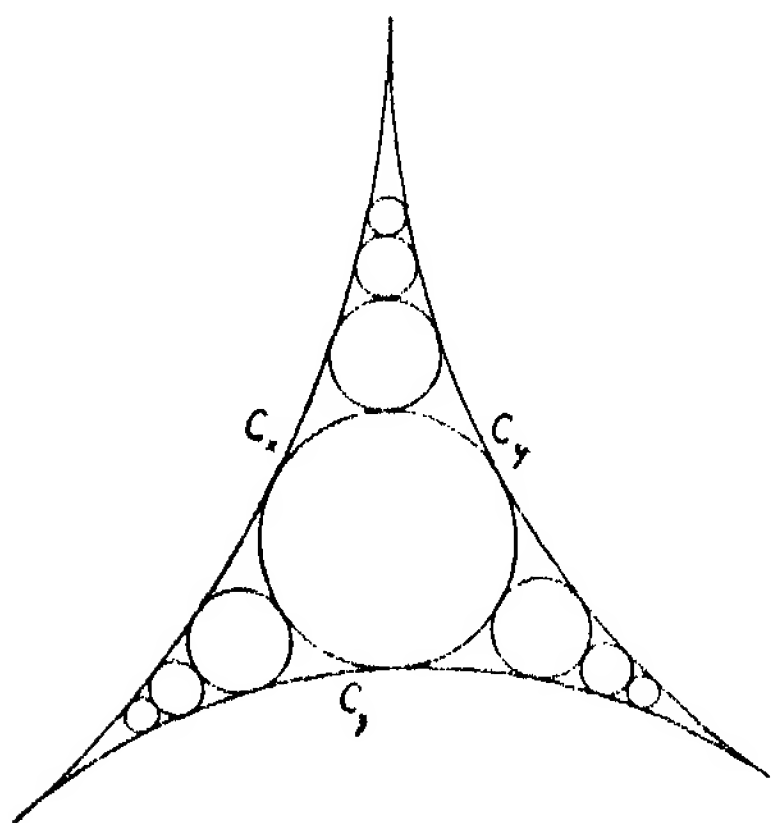


FIGURE 2

then the circles of the n th subdivision are

$$C(C(a_1, b, c), b, c), C(C(a_2, a, c), a, c), C(C(a_3, a, b), a, b).$$

2. *The Fundamental Lemma.* We now establish the following

LEMMA: *A necklace packing of a curvilinear triangle $T(x, y, z)$ covers more than one-half the area of $T(x, y, z)$.²*

Let us effect a necklace packing of $T(x, y, z)$. Let U and V denote any two tangent circles of the necklace set, with radii u, v and centers s_u, s_v , respectively. We connect s_u to s_v with a line segment. We also draw line segments from the points s_u, s_v to the points x_u, x_v at which U and V touch one of the sides of $T(x, y, z)$, say C_x (see Fig. 3). We note that

$$\angle x_u s_u s_v +$$

$$\angle x_v s_v s_u \leq \pi. \quad (1)$$

Let $K[R]$ denote the area of the region R . We show that $K[T(u, v, x)]$ is less than the sum of the areas of the sectors S_u, S_v of U and V included in the triangle (s_u, s_v, s_x) , where s_x is the center of C_x . Let $LT(u, v, x)$ denote the linear triangle joining the vertices of the curvilinear triangle $T(u, v, x)$. Then

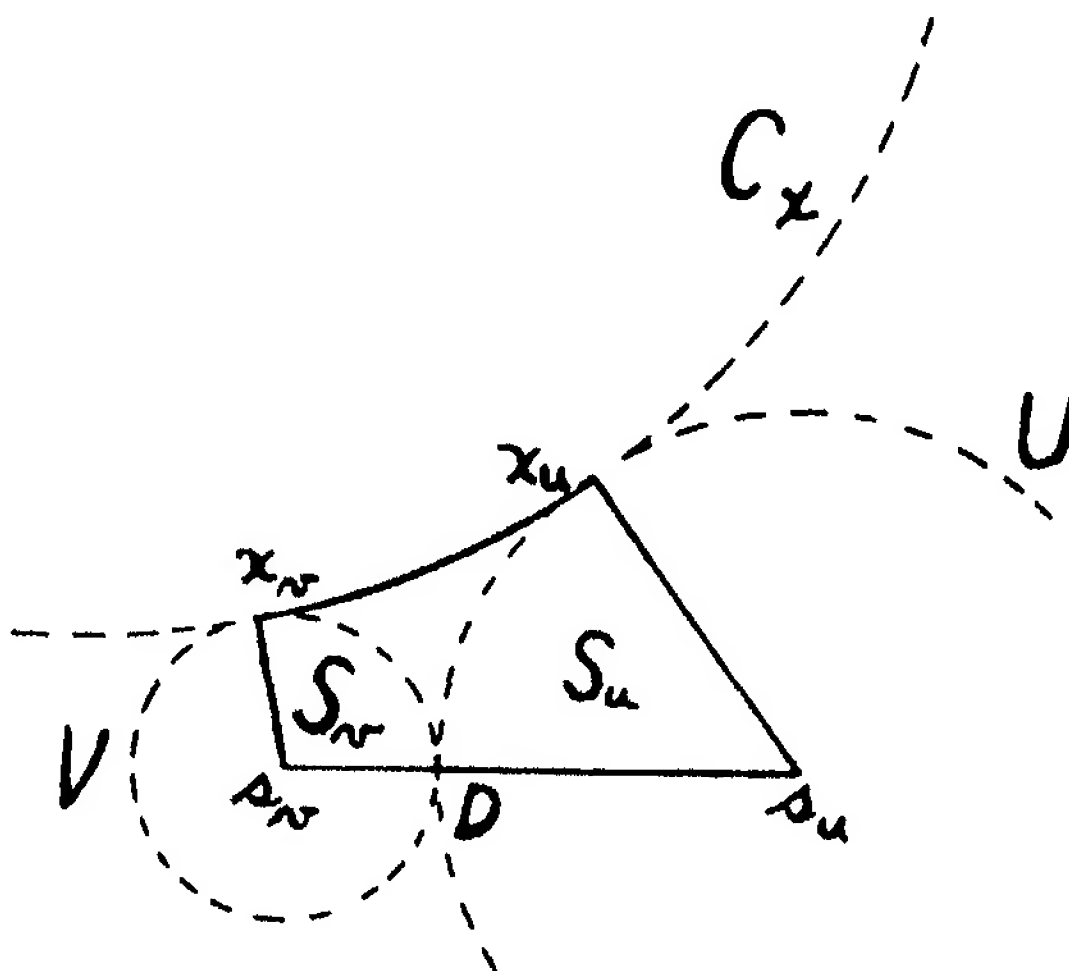


FIGURE 3

$$K[T(u, v, x)] < K[LT(u, v, x)]. \quad (2)$$

Now $LT(u, v, x)$ divides triangle (s_u, s_v, s_x) into four mutually exclusive triangles. We show that $K[LT(u, v, x)]$ is less than the sum of the areas of those two of the other three triangles which contain the points s_u and s_v respectively.

Let us consider the set of triangles with vertices R, S and T , where the points R and S are fixed, the segment \overline{RS} being of constant length k , and where T may be any point in one of the half planes determined by the line going through R and S (see Fig. 4). We also admit the case where $\angle RTS$ is zero, or $RT \parallel TS$. Let P be any point on \overline{RS} not an end-point. Let the segment $\overline{RP} = \alpha$ and the segment $\overline{PS} = \beta$. We lay off a segment \overline{RQ} equal to α or \overline{RT} and a segment \overline{SW} equal to β on \overline{ST} . We show that

the area δ of ΔPQW is less than or equal to the sum μ of the areas of ΔRPQ and ΔPWS .³ Now

$$\delta = 2\alpha\beta \sin(R/2) \sin(S/2) \sin((R+S)/2) \quad (3)$$

and

$$\mu = (\alpha^2/2) \sin R + (\beta^2/2) \sin S. \quad (4)$$

We must show that $\delta \leq \mu$ for

$$\angle R + \angle S \leq \pi. \quad (5)$$

From (5) we have

$$\sin(S/2) \leq \sin((\pi - R)/2) = \cos(R/2) \quad (6)$$

and

$$\sin(R/2) \leq \sin((\pi - S)/2) = \cos(S/2). \quad (7)$$

Let us first consider the case where

$$\sin R \leq \sin S. \quad (8)$$

Then from (6) and (8) we have

$$\begin{aligned} & 2\alpha\beta \sin(R/2) \sin(S/2) \sin((R+S)/2) \\ & \leq 2\alpha\beta \sin(R/2) \sin(S/2) \\ & \leq 2\alpha\beta \sin(R/2) \cos(R/2) \\ & = \alpha\beta \sin R \\ & \leq ((\alpha^2 + \beta^2)/2) \sin R \\ & = (\alpha^2/2) \sin R + (\beta^2/2) \sin R \\ & \leq (\alpha^2/2) \sin R + (\beta^2/2) \sin S. \end{aligned} \quad (9)$$

Now let us consider the case where

$$\sin S < \sin R. \quad (10)$$

This case may be established by a sequence of inequalities as in (9), if we now use (7) where we have used (6), and (10) where we have used (8).

Now let D be the point at which the circles U and V are tangent. Then as a consequence of the latter argument we have

$$\begin{aligned} K[T(u, v, x)] & < K[LT(u, v, x)] \leq K[\Delta(s_u, D, x_u)] + K[\Delta(s_v, D, x_v)] \\ & < K[S_u] + K[S_v]. \end{aligned} \quad (11)$$

Now let $T(x, y, z)$ be completely subdivided as above. Thus, we join the centers of any two tangent circles of the necklace set associated with $T(x, y, z)$ with linear segments. And, if W is any circle of this necklace

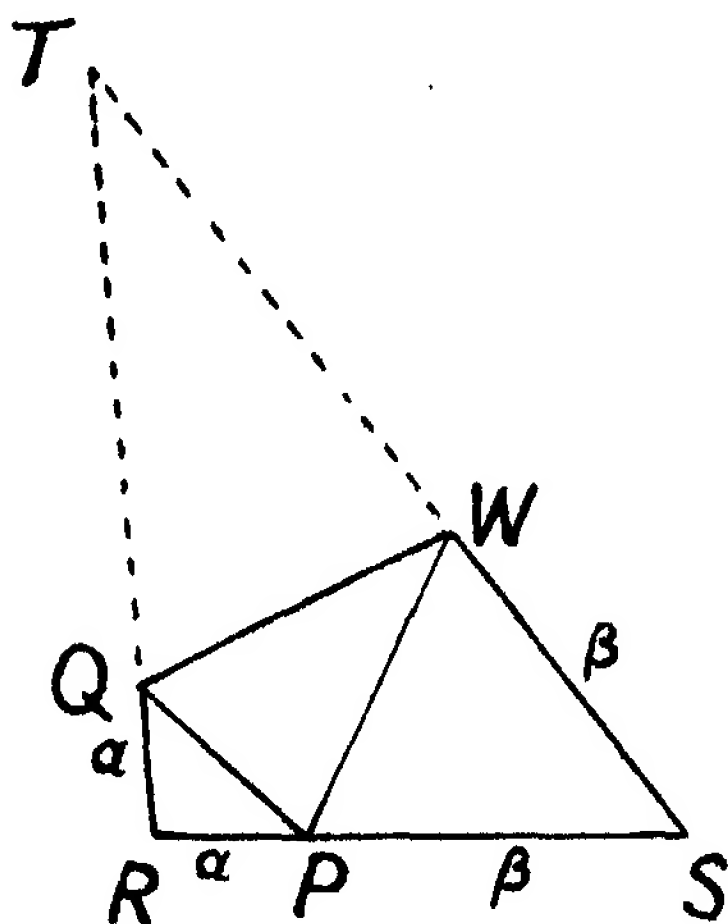


FIGURE 4

set, then we join its center to the points where W contacts C_x , C_y or C_z , with linear segments. Thus, $T(x, y, z)$ is the sum of a denumerable set of non-overlapping regions $\{A_i\}$, each bounded by three linear segments and a subarc of C_x , C_y or C_z (see solid line in Fig. 3). Let S_{i1} and S_{i2} be the two circular sectors included in A_i , and l_i the remaining curvilinear triangle. To each A_i we may now apply (11). Then

$$\begin{aligned} K[T(x, y, z)] &= \sum_{i=1}^{\infty} K[A_i] \\ &= \sum_{i=1}^{\infty} (K[S_{i1}] + K[S_{i2}] + K[l_i]) \\ &= \sum_{i=1}^{\infty} (K[S_{i1}] + K[S_{i2}]) + \sum_{i=1}^{\infty} K[l_i] \\ &\geq 2 \sum_{i=1}^{\infty} K[l_i]. \end{aligned} \quad (12)$$

Therefore,

$$\left(\sum_{i=1}^{\infty} K[l_i] \right) / K[T(x, y, z)] \leq 1/2. \quad (13)$$

This proves our fundamental lemma.

3. *Proof of the Theorem.* Let us effect a necklace packing of our curvilinear triangle $T(a, b, c)$. Let $\omega = K[T(a, b, c)]$. Denote the sum of the areas of the circles of this necklace set by σ_1 . Then the area of the vacancy v_1 is

$$\omega - \sigma_1 \leq \omega/2. \quad (14)$$

But v_1 consists of a denumerable set of curvilinear triangles. In each of these effect a necklace packing and let σ_2 denote the sum of the areas of all these necklace sets. Then the area of our second vacancy v_2 is

$$\omega - (\sigma_1 + \sigma_2) \leq \omega/2^2. \quad (15)$$

Proceeding similarly, the area of the n th vacancy v_n is

$$\omega - \sum_{i=1}^n \sigma_i \leq \omega/2^n \quad (16)$$

and

$$\lim_{n \rightarrow \infty} \omega - \sum_{i=1}^n \sigma_i = 0. \quad (17)$$

But $\sum_{i=1}^{\infty} \sigma_i$ is the sum of the areas of the Apollonian packing of $T(a, b, c)$.

Thus the area of the vacancy of the Apollonian packing of $T(a, b, c)$ is zero; or, the sum of the areas of the circles of an Apollonian packing in $T(a, b, c)$ is equal to the area of $T(a, b, c)$. Or, if we borrow the geological

term *porosity*, and let it designate the ratio of the area of the vacancy (relative to a set of mutually external circles in $T(a, b, c)$) to the area of $T(a, b, c)$, then we may assert that the porosity of an Apollonian packing of circles in $T(a, b, c)$ is zero.

The packing of spheres requires methods which are essentially different from that of the present two-dimensional discussion. This question will be the subject of another paper.

In the case of the best covering of the plane with equal circles (no matter how small), the covering ratio is 0.9069, and thus the porosity is 0.0931. The covering ratio of space with equal spheres (no matter how small) is 0.7404 and thus the porosity is 0.2596 (normal packing). This is well known in geological literature in connection with the packing of sand and the amount of oil contained in the oil sands. Our work deals with the packing of unequal spheres, and we find that the porosity may be made as small as we please.⁴

¹ Kasner, E., Comenetz, G., and Wilkes, J., "The Covering of the Plane by Circles," *Scripta Mathematica*, 9, 19-25 (1943).

² We have good reason to believe that the ratio of the sum of the areas of the circles of a necklace packing in any $T(x, y, z)$, to the area of $T(x, y, z)$, is greater than $\pi/4 = 0.7854$ and less than 0.8223. This problem will be treated in another paper. We thank Aida Kalish and Hüseyin Demir for assistance in the calculation of the upper limit 0.8223.

³ We note that this part of the proof is somewhat more general than that actually required by the lemma.

⁴ Kasner, E., "Note on Non-Apollonian Packing in Space," *Scripta Mathematica*, 9, 26 (1943). See also *Science*, Oct. 16, 1942. Incidentally we note the following new theorem: In any circular triangle (bounded by mutually tangent circles) the conformal bisectors of the three horn angles (these are necessarily circles) are concurrent. The resulting point we call the *inversive center* of the triangle. For the bisection of analytic horn angles see the senior author's papers on conformal geometry.

CONCERNING CONTINUA WHICH HAVE DENDRATOMIC SUBSETS

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The subset K of the compact continuum M is said to be a *dendratomic* subset of M if there exists an upper semicontinuous collection G of mutually exclusive continua filling up M such that (1) K is an element of G , (2) G is a dendron with respect to its elements and (3) if H is an upper semicontinuous collection of mutually exclusive continua filling up M such

that H is a dendron with respect to its elements then every element of G is a subset of some element of H .

The compact continuum M will be called a *web* provided there exist two upper semicontinuous collections H_1 and H_2 of mutually exclusive continua such that (1) each of these collections fills up M , (2) each of them is a dendron with respect to its elements and (3) there exists an uncountable subcollection W of the collection H_1 such that no element of W is a subset of any element of H_2 . A compact continuum is said to be *webless*¹ if it contains no web.

THEOREM 1. *If the compact continuum M is a web there exist two upper semicontinuous collections Z_1 and Z_2 of mutually exclusive continua such that (1) for each i ($i = 1, 2$), Z_i fills up M and is an arc with respect to its elements, and (2) every continuum of Z_1 intersects every continuum of Z_2 .*

Proof. Let H_1 , H_2 and W denote collections satisfying, with respect to M , all of the conditions stated in the above definition of a web. There exist two mutually exclusive closed point sets L and T and an uncountable subcollection W_1 of W such that every continuum of the collection W_1 intersects both L and T . There exists a sequence γ of continua of the collection W_1 which converges to a continuum a_1 of that collection. There exist two points A and B belonging to a_1 but not to the same continuum of the collection H_2 . Let α_2 and β_2 denote the continua of H_2 that contain A and B , respectively. Since H_2 is a continuous curve with respect to its elements there exists an arc $\alpha_2\beta_2$ of elements of H_2 having α_2 and β_2 as its end-elements. Since H_2 fills up M and is a dendron with respect to its elements, the continua α_2 and β_2 are separated from each other in M by every continuum of the collection $\alpha_2\beta_2$ other than themselves. Let a_2 denote a continuum of the collection $\alpha_2\beta_2$ distinct from α_2 and β_2 , let b_2 denote a continuum of this collection that separates a_2 from β_2 in M and let a_2b_2 denote the arc of elements of $\alpha_2\beta_2$ whose end-elements are a_2 and b_2 . Let D_A denote the set of all points of M that are separated from b_2 in M by a_2 and let D_B denote the set of all those that are separated from a_2 in M by b_2 . The point sets D_A and D_B are open subsets of M containing A and B , respectively. Since γ converges to a_1 there exists a continuum b_1 belonging to γ , distinct from a_1 and intersecting both D_A and D_B . There exists an arc a_1b_1 of elements of H_1 having a_1 and b_1 as its end-elements.

For each i ($i = 1, 2$), let Z_i denote the collection of all continua z such that, for some continuum x of a_ib_i , z is the sum of all continua y of H_i such that y is not separated from x in M by any continuum of the collection a_ib_i .

THEOREM 2. *In order that a compact continuum should have dendratomic subsets it is necessary and sufficient that it should not be a web.*

Proof. If the statement of theorem 35 of page 360 of chapter V of my book, "Foundations of Point Set Theory,"¹ is strengthened by the substitution of "compact continuum which is not a web" in place of "web-

less compact continuum," the resulting theorem may be proved by an argument identical with the one there given to prove theorem 35 except for the substitution of "not a web" for "webless" in its tenth line. With the help of theorem 37 of that chapter it follows that if a compact continuum is not a web then it has dendratomic subsets.

Suppose, now, that the compact web M has dendratomic subsets. Let Z_1 and Z_2 denote collections satisfying, with respect to M , all of the conditions of theorem 1 of the present paper. For each i ($i = 1, 2$), let x_i and y_i denote two continua of the collection Z_i . By hypothesis there exists an upper semicontinuous collection G of mutually exclusive continua filling up M such that (1) G is a dendron with respect to its elements and (2) if a continuum of the collection G intersects a continuum which belongs either to Z_1 or to Z_2 then it lies wholly in that continuum. Let a and b denote continua of the collection G which are subsets of $x_1 \cdot x_2$ and $x_1 \cdot y_2$, respectively. Let U denote the collection of all continua of G that are subsets of x_1 and let V denote the collection of all those that are subsets of the continuum $x_2 + y_1 + y_2$. With respect to the elements of G , U and V are intersecting subcontinua of G whose common part is the set of all elements of G that are subsets either of $x_1 \cdot x_2$ or of $x_1 \cdot y_2$ and clearly this set is not a connected set of elements. But G is a dendron with respect to its elements and no dendron contains two intersecting continua whose common part is not connected. Thus the supposition that M has dendratomic subsets leads to a contradiction.

Definition. The compact continuum M is said to be a *triad*³ if it is the sum of three continua such that the common part of all three parts of them is both a non-vacuous subcontinuum of each of them and the common part of every two of them.

THEOREM 3. *In order that the compact continuum M should be a triad it is necessary and sufficient that it should contain a continuum K such that $M - K$ has more than two components.*

Proof. This condition is clearly necessary. It is also sufficient. For if K is a subcontinuum of M such that $M - K$ has at least three components then (1) by a theorem of F. B. Jones, $M - K$ is the sum of three mutually separated point sets T_1 , T_2 and T_3 and (2) the point sets $K + T_1$, $K + T_2$ and $K + T_3$ are continua and K is the common part of every two of them.

THEOREM 4. *The compact continuum M is a triad if it contains three continua α , β and γ such that (1) α and β are mutually exclusive, (2) α separates M , and (3) γ intersects α and separates two points of β from each other in M .*

Proof. By hypothesis, $M - \gamma$ is the sum of two mutually separated point sets T_1 and T_2 such that $T_1 \cdot \beta$ and $T_2 \cdot \beta$ exist and $M - \alpha$ is the sum of two mutually separated point sets H and K such that H contains β .

The point sets $T_1 \cdot H + \alpha + \gamma$, $T_2 \cdot H + \alpha + \gamma$ and $K + \alpha + \gamma$ are continua, their sum is M and the common part of every two of them is the continuum $\alpha + \gamma$.

It is not true that the compact continuum M is a triod if it contains two continua β and γ such that β separates two points of γ from each other in M and γ separates two points of β from each other in M . If the continuum M is the sum of three arcs AXB , AYB and AZB such that the common part of every two of them is $A + B$ then M contains two such continua β and γ . But it is not a triod.

THEOREM 5. *Every web is a triod.*

Proof. Suppose M is a web. Let Z_1 and Z_2 denote two upper semicontinuous collections satisfying, with respect to M , all of the conditions listed in the statement of theorem 1. Let α and β denote two continua belonging to but not end-elements of, the collection Z_1 and let γ denote one belonging to Z_2 but distinct from its end-elements. The continuum γ intersects both α and β and $M - \gamma$ is the sum of two mutually separated point sets H and K . Let X and Y denote points belonging to H and K , respectively, and let h and k denote the continua of the collection Z_2 that contain X and Y , respectively. The continua h and k are subsets of H and K , respectively. But β intersects both of these continua. Thus $\beta \cdot H$ and $\beta \cdot K$ exist. The continuum γ separates $\beta \cdot H$ from $\beta \cdot K$ in M . Therefore, by theorem 4, M is a triod.

THEOREM 6. *If the compact continuum M is not a triod it has dendratomic subsets and they are arcatomic³ subsets of M .*

Proof. That M has dendratomic subsets follows directly from theorems 2 and 5. With the aid of the fact that every compact dendron which is not an arc is a triod it follows that if an upper semicontinuous collection of mutually exclusive continua fills up M and is a dendron with respect to its elements then it is an arc with respect to its elements. Hence the dendratomic subsets of M are arcatomic subsets of it.

THEOREM 7. *If A and B are two end-points of the compact dendron M there exists an upper semicontinuous collection H of mutually exclusive continua filling up M such that, with respect to its elements, H is an arc whose end-elements are A and B .*

Proof. The dendron M contains an arc AB . For each point P of AB , let h_P denote P , or the continuum obtained by adding to P every component of $M - AB$ of which P is a limit point, according as there does not or does exist at least one such component. Let H denote the collection of all such continua h_P . This collection is upper semicontinuous, it is an arc with respect to its elements and its end-elements are h_A and h_B . But $h_A = A$ and $h_B = B$.

THEOREM 8. *If a compact continuum M has arcatomic subsets then not only are they dendratomic subsets of M but every upper semicontinuous collec-*

tion of mutually exclusive continua which fills up M and which is a non-degenerate dendron with respect to its elements is also an arc with respect to its elements.

Proof. Let G denote the collection of all arcatomic subsets of M . Suppose H is an upper semicontinuous collection of mutually exclusive continua filling up M such that H is a dendron with respect to its elements. Suppose this dendron has three end-elements, α , β and γ . With the help of Theorem 7 and certain theorems of the above-mentioned book it may be seen that there exists an upper semicontinuous collection Q of mutually exclusive continua filling up M such that (1) every continuum of H is a subset of some continuum of Q , (2) α and β belong to Q , and (3) with respect to its elements Q is an arc whose end-elements are α and β . It follows that each of the continua α , β and γ contains every continuum of the collection G which it intersects. But if a , b and c denote the set of all the continua of G that lie in α , the set of all those that lie in β and the set of all those that lie in γ , respectively, then, with respect to the elements of G regarded as points, a , b and c are three mutually exclusive subcontinua of G and $G - a$, $G - b$ and $G - c$ are all connected. This is contrary to the fact that G is an arc with respect to its elements. Hence H has only two end-elements. Therefore it is an arc with respect to its elements. It follows that every element of G is a dendratomic subset of M .

It is not true that if a compact continuum has arcatomic subsets then it is not a triod.

Example. In a Euclidean plane let AB denote a straight line interval and let T denote a totally disconnected perfect subset of AB containing both A and B . Let Q_1, Q_2, Q_3, \dots denote the components of $AB - T$. For each n let A_n and B_n denote the end-points of the segment Q_n , A_n being between A and B_n . For each point P of $T - A$ let J_P denote the circle with center at A which passes through P . For each positive integer n let α_n denote a set of n straight line intervals $A_{1n}B_{1n}, A_{2n}B_{2n}, \dots, A_{nn}B_{nn}$ such that the points $A_{1n}, A_{2n}, \dots, A_{nn}$ are equally spaced on JA_n and $B_{1n}, B_{2n}, \dots, B_{nn}$ are on JB_n and, for each i ($1 \leq i \leq n$), A_{in} lies between B_{in} and A . Let K_n denote the continuum obtained by adding together JA_n, JB_n and all the intervals of the set α_n . Let K denote the point set $A + K_1 + K_2 + K_3 + \dots$. Let G denote the collection of all continua g such that either g is A or, for some n , g is K_n or, for some point P of $T - K \cdot T$, g is J_P . Let M denote the sum of all the continua of the collection G . The point set M is a continuous curve and G is an arc with respect to its elements and its elements are arcatomic subsets of M . But M is a triod.

THEOREM 9. *Every compact irreducible continuum between two points has dendratomic subsets and they are also its arcatomic subsets.*

Theorem 9 is a consequence of theorem 6 and the fact that no irreducible continuum between two points is a triod.

¹ See page 357 of my book "Foundations of Point Set Theory," *Am. Math. Soc. Colloquium Pub.*, Vol. XIII, New York (1932).

² See my paper, "Concerning Triodic Continua in the Plane," *Fundamentae Mathematicae*, 13, 262 (1929) and R. H. Sorgenfrey's as yet unpublished doctoral dissertation titled "Concerning Triodic Continua."

³ An arcatomic subset of M will be defined if, in the above definition of a dendratomic subset of M , "dendron" is replaced by "arc" and "dendratomic" is replaced by "arcatomic."

CONCERNING WEBS IN THE PLANE

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In my paper¹ "Concerning Continua Which Have Dendratomic Subsets," I defined webs and webless continua. As may be seen from the following example, it is not true that every continuum which is not a web is webless.

Example. In Euclidean space of three dimensions let E denote a plane, let J denote a circle lying in E , let I denote the bounded component of $E - J$ and let K denote $I + J$. Let T denote a connected point set topologically equivalent to a ray and such that T and E are mutually exclusive but $\bar{T} = T + K$. The point set \bar{T} is an irreducible continuum from K to some point not lying in E . This continuum is not a web. But it contains the web K .

However, the following theorem holds true.

THEOREM 1. *In the plane, if a compact continuum contains a web then it is a web.*

Proof. In a Euclidean space Σ of two dimensions suppose M is a compact continuum containing a web L . There exist two upper semicontinuous collections H_1 and H_2 of mutually exclusive continua such that each of these collections fills up L and such that each of them is an arc with respect to its elements and every continuum of H_1 intersects every continuum of H_2 .

Suppose t is a component of the common part of a continuum x_1 of the collection H_1 and a continuum x_2 of the collection H_2 . Let y_1 denote a continuum of H_1 distinct from x_1 and let y_2 denote a continuum of H_2 distinct from x_2 . Since y_1 intersects y_2 , $y_1 + y_2$ is a continuum. Clearly this continuum is a subset of $L - t$. Let N denote $L - (x_1 \cdot x_2)$. Suppose P is a point of N . There exists a continuum p containing P , lying in N and belonging either to H_1 or to H_2 . If p belongs to H_1 it intersects y_2 and if it belongs to H_2 it intersects y_1 . Hence in either case it intersects $y_1 + y_2$.

and therefore $p + y_1 + y_2$ is a connected subset of N containing P . It follows that N is connected. But every component of a proper closed subset of a compact continuum contains a limit point of that continuum minus that subset. Hence every component of $x_1 \cdot x_2$ contains a limit point of the connected point set N . Therefore $L - \iota$, the sum of N and all such components other than ι (if there are any) is connected.

For each component ι of the common part of a continuum of H_1 and a continuum of H_2 , let h_ι denote ι plus the set of all points X of M , if there are any, such that ι separates X from $L - \iota$. Let H denote the set of all such continua h_ι . For each i ($i = 1, 2$) let H'_i denote the set of all sets h' such that, for some element h of H_i , h' is the set of all h_ι 's for all ι 's which are subsets of h . Let S' denote the collection of all continua P' such that P' is either a continuum of the collection H or a point which neither belongs to any continuum of H nor is separated by any continuum of H from any other continuum of H . If the elements of S' are regarded as "points" and the subcollection Z of S' is regarded as a "region" if and only if Z^* is a domain with respect to $(S')^*$ then Axioms 0, 1', C, 2, 3, 4 and 5 of my book² *Foundations of Point Set Theory* all hold true for the space Σ_1 so obtained and, in this space, H is a continuous curve with no cut point and there exist a simple domain I , a simple closed curve J , four points A, B, C and F and two collections G_1 and G_2 satisfying all the requirements of the hypothesis of theorem 9 of page 380 and such that, for each i ($i = 1, 2$) every continuum of the collection G_i is a subcontinuum of some continuum of the collection H'_i . By a modification³ of the argument given on pages 380 and 381 to prove theorem 9 it may be shown that, in the space Σ_1 , there exists an upper semicontinuous collection G of mutually exclusive continua such that (a) if the elements of G are regarded as points and the subcollection Z of G is regarded as a region if and only if Z^* is a domain with respect to G^* then the space Σ_2 so obtained satisfies Axioms 0, 1', C, 2, 3, 4 and 5, (b) in Σ_1 every non-degenerate continuum of the collection G is a subset of H plus the set of all elements of S' which are points of M which in Σ belong to no continuum of the collection H , (c) if, in Σ_1 , g is a continuum of the collection G and P is an element of S' which either belongs to H or is, in Σ , a point of M which belongs to no continuum of the collection H then, in Σ_1 , g does not separate P from any other continuum of the collection G , (d) if P is an element of S' which either belongs to H or is a point of M which, in Σ , belongs to no continuum of H then P either belongs to G or is, in the space Σ_1 , an element of some continuum of the collection G , and (e) in the space Σ_2 there exists a connected domain D with a connected boundary β such that every degenerate element of G that belongs to D is, in Σ , a point of M or a continuum of the collection H . In the space Σ_2 let W denote the collection whose degenerate elements are the points of D and whose only non-degenerate element is the continuum γ obtained by adding to β

all points of Σ_2 which do not belong to D and which in Σ_1 are points of H or points which in Σ belong to M but to no continuum of the collection H . In Σ_2 , W is an upper semicontinuous collection of mutually exclusive continua. There exists a reversibly continuous transformation throwing the continua of the collection W into the points of a sphere in a Euclidean space E of three dimensions. But clearly a sphere in E is a web. It follows that W is a web with respect to its elements. Hence, in the space Σ_2 , there exist two upper semicontinuous collections of mutually exclusive continua Z_1 and Z_2 such that (1) each of these collections fills up $D + \gamma$, (2) each of them is an arc with respect to its elements, (3) each element of Z_1 intersects each element of Z_2 and (4) one element of Z_1 contains γ . For each continuum z of the collection Z_i ($i = 1, 2$) let l_i denote the continuum in the space Σ_1 obtained by adding together all the continua in that space which are points of z in the space Σ_2 and let t_i denote the continuum in the space Σ obtained by adding together all the continua in the space Σ which are points of l_i in the space Σ_1 . Let T_i denote the collection of all such t_i 's for all elements z of Z_i . In the space Σ , for each i , T_i is an upper semicontinuous collection of mutually exclusive continua filling up M , T_i is an arc with respect to its elements and every continuum of the collection T_1 intersects every one of the collection T_2 . Hence M is a web.

THEOREM 2. *If in the plane, M is a compact continuum, G is an upper semicontinuous collection of mutually exclusive continua filling up M and H is another such collection and, for every two continua g and h belonging to G and H , respectively, $g \cdot h$ exists and is totally disconnected then each of the collections G and H is a continuous curve with respect to its elements.*

Proof. Since every continuum of the collection G intersects every one of the collection H , therefore there exists a positive number ϵ such that every continuum belonging either to G or to H is of diameter more than ϵ .

Suppose M is not a continuous curve. With the help of Theorem 8 of chapter II it may be shown that there exist a sequence M_1, M_2, M_3, \dots of mutually exclusive subcontinua of M and a simple closed curve J containing four points A, B, C and D such that $A + C$ separates B from D on J and such that if I denotes the interior of J and, for every two points X and Y of this set of four, XY denotes the arc of J from X to Y which contains neither of the remaining ones then (1) the diameter of J is less than ϵ , (2) for each n , M_n is a component of $M \cdot \bar{I}$ and M_{n+1} separates AD from M_n in \bar{I} . Let C_1D_1 denote an arc lying wholly in I except for its end-points C_1 and D_1 which lie between B and C and between A and D , respectively, on the arcs BC and AD . Let H' denote the set of all point sets x such that x is a component of the common part of \bar{I} and some continuum of the collection H . For each n , M_n contains a point P_n of C_1D_1 and there exists a continuum h'_n belonging to H' and containing P_n and therefore lying in M_n . For each n , h'_n intersects either AB or CD . It follows that there exist two points

A_1 and B_1 and an infinite ascending sequence of positive integers n_1, n_2, n_3, \dots such that (1) either A_1 is D and B_1 is C or A_1 is A and B_1 is B and (2) for every j , h'_{n_j} intersects A_1B_1 . Let I_1 denote the component of $I - I \cdot (C_1D_1)$ whose boundary contains the arc A_1B_1 . Let C_2D_2 denote an arc lying wholly in I_1 except for its end-points C_2 and D_2 which lie between B_1 and C_1 and between A_1 and D_1 , respectively, on the arcs BC and AD . Let G' denote the set of all point sets x such that x is a component of the common part of \bar{I}_1 and some continuum of the collection G . There exist points A_2 and B_2 and an infinite ascending subsequence m_1, m_2, m_3, \dots of the sequence n_1, n_2, n_3, \dots such that (1) either A_2 is D_1 and B_2 is C_1 or A_2 is A_1 and B_2 is B_1 , (2) for each j there is a continuum g_{m_j} belonging to G' and intersecting both A_2B_2 and C_2D_2 and lying in M_{m_j} . Let I_2 denote the component of $I_1 - I_1 \cdot (C_2D_2)$ whose boundary contains A_2B_2 and C_2D_2 . For each j there exist two continua g_j and h_j such that (1) each of them intersects both A_2B_2 and C_2D_2 , (2) g_j is a component of $\bar{I}_2 \cdot g'_{m_j}$ and h_j is a component of $\bar{I}_2 \cdot h'_{m_j}$. The sequence g_1, g_2, g_3, \dots converges to a continuum L and h_1, h_2, h_3, \dots converges to the same continuum. Hence there exist two continua g and h , belonging to G and H , respectively, such that L is a subset of each of them. But, since it intersects both A_2B_2 and C_2D_2 , L is non-degenerate. This involves a contradiction. Hence M is a continuous curve. Therefore G is a continuous curve with respect to its elements and so is H .

If, in the statement of theorem 2, the words "in the plane" are replaced by "in Euclidean space of three dimensions," the resulting proposition is false.

Example. In Euclidean three dimensional space let AB denote a straight-line interval of length 1, let K denote a cube having AB as one of its edges and let F and L denote the two faces of K that contain AB . Let T denote the point set consisting of K plus its interior. For each n , let F_n denote the common part of T and a plane parallel to and at a distance equal to $1/n$ from, the plane in which F lies. Let M denote the continuum $F + L + F_1 + F_2 + F_3 + \dots$. Let G denote the collection of all straight-line intervals g of length 1 lying in M such that g either coincides with AB or lies on a straight line parallel to the one containing AB . Let H denote the collection of all continua h such that h is the common part of M and some plane perpendicular to AB . The collections G and H are upper semicontinuous, each of them fills up M and if g and h are continua belonging to G and H , respectively, $g \cdot h$ is a single point. The collection H is an arc¹ with respect to its elements. But G is not a continuous curve with respect to its elements and M is not a continuous curve.

¹ These PROCEEDINGS, 29, 384-389 (1943).

² Amer. Math. Soc. Colloquium Pub., New York (1932). References in this article to pages and chapters are to pages and chapters of this book. In the present connection see page 382.

* This argument is to be modified as follows: After the second sentence interpolate "Suppose either X denotes A and Y denotes C or X denotes B and Y denotes F . If X belongs to T let r_X denote XY plus the continuum of the collection G that contains X . If the interval l of the collection T has one end-point at X , let r_T denote $l + x_l + y_l$, where x_l is the continuum of the collection T that contains the other end-point of l and y_l is either XY or XY plus the continuum of G_l that intersects XY according as XY and G_l^* are or are not mutually exclusive." In the third sentence, after " T ", interpolate "distinct from A and from B ." Instead of the sentence beginning in the 18th line of page 381, write "In the space Σ_1 , for each continuum q of the collection Q , let g_q denote the boundary of q , plus the set of all points of q , if there are any, which either (a) belong to H or (b) are points of M which in Σ belong to no continuum of the collection H . Let G denote the collection of all such g_q 's."

⁴ See theorem 16 of chapter V.

⁵ Let M denote a continuum formed by a sequence of right pyramids all with the same square base α , their vertices converging to that of the outermost one. Let l_1 and l_2 denote two adjacent sides of α and let H_i denote the set of all intersections of M with planes perpendicular to l_i . Both H_1 and H_2 are arcs with respect to their elements but M is not a continuous curve.

EFFICIENT COMPUTATION OF THE LATENT VECTORS OF A MATRIX

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In statistics, in quantum mechanics and in the study of dynamical oscillations it is often necessary to compute the latent roots and vectors of a matrix. A variety of methods are available for this purpose, involving direct algebraic computation, iteration and the application of perturbation-variation methods. It is the author's tentative conclusion from experience that the last two methods are excellent if only a few latent roots and vectors are desired, say those corresponding to the lowest or highest few roots, or if there exists some *a priori* familiarity with the data which permits very good initial guesses to be made. But in the general case of high order matrices both the iteration methods and the perturbation-variation methods become tedious, and so recourse must be had to direct algebraic computations.

A variety of methods are available under this heading, and the computer will choose between them not on the basis of their adequacy on constructed text-book examples, but in terms of a careful count of the number of calculations involved in each. Every method must involve the solution of a polynomial of the n th degree; but in addition, all of the methods known

to the present writer seem to involve multiplications which increase with the fourth power of n , where the matrix involved is of order n by n . It is the purpose of this note to present, perhaps for the first time, a method which gives latent vectors as well as latent roots after multiplications which increase with the third power of n .

1. *Description of Procedure.* Let a be the n by n matrix in question, and let h be an arbitrary column vector. Form the matrix products $(h_0, h_1, h_2, \dots, h_n)$ by means of the operations $[Ih, ah, a(ah), \dots, a(a^{n-1}h)]$. Then in consequence of the Cayley-Hamilton theorem that a matrix satisfies its own characteristic equation, we derive the coefficients of the characteristic equation $(1, p_1, p_2, \dots, p_n)$ by solution of the following n linear equations

$$[h_0, h_1, \dots, h_{n-1}] \begin{bmatrix} p_{n-1} \\ p_{n-2} \\ \vdots \\ p_1 \end{bmatrix} = -h_n.$$

Then let the characteristic equation, $f_n(X) = \sum_0^n p_j X^{n-j} = 0$, be solved by any method for the latent roots (X_1, X_2, \dots, X_n) , assumed for simplicity to be distinct. We now form new polynomials by the relations

$$\begin{aligned} f_{n-1}(X) &= X^{n-1} + p_1 X^{n-2} + \dots + p_{n-1} \\ f_{n-2}(X) &= X^{n-2} + p_1 X^{n-3} + \dots + p_{n-2} \\ &\vdots \\ f_1(X) &= X + p_1 \\ f_0(X) &= 1 \end{aligned}$$

where each is formed from the previous by dropping off the last term and lowering the degree of the remaining terms. Expressions of the form $f_i(X_j)$ are easily computed as partial remainders in the familiar process of synthetic division.

Then the n latent vectors of a , (V_1, V_2, \dots, V_n) , can be shown to be given by the product of the following two square matrices

$$V = [h_0, h_1, h_2, \dots, h_{n-1}] [f_{n-1}(X_j)]. \quad (1)$$

Should the original column vector, h , have been a linear combination of less than n latent vectors, the above process will fail; however, the probability of this occurring is very small, and such occurrences can easily be detected and allowed for. There is no reason why complex latent roots

and vectors cannot be handled in the above process. In the important special case where a is symmetrical, only real quantities will be involved, and a check upon the numerical computations is provided by the conjugate property $V_j' V_i = 0$, for $i \neq j$. When repeated roots are encountered, the modifications are relatively minor.

The labor involved in numerical processes of the above type is best reckoned in terms of the required number of multiplications. The method described here involves multiplications of the order $8n^3/3$, or about the equivalent of three square matrix multiplications. In addition, one n th degree polynomial must be solved. It will be noted that each latent vector can be determined independently of all the rest, once its corresponding latent root has been determined. Approximate values of a latent vector can be computed from the insertion of approximate roots in the process of synthetic division indicated above.

2. *Proof.* Consider the system of differential equations written in matrix form

$$DY(t) = aY(t).$$

If the latent roots are all distinct, it is known that the solution of these equations for initial values $Y(0) = h$ is unique and given by

$$\begin{aligned} Y_1(t) &= c_{11} \exp X_1 t + c_{12} \exp X_2 t + \dots + c_{1n} \exp X_n t \\ Y_2(t) &= c_{21} \exp X_1 t + c_{22} \exp X_2 t + \dots + c_{2n} \exp X_n t \\ &\vdots \\ Y_n(t) &= c_{n1} \exp X_1 t + c_{n2} \exp X_2 t + \dots + c_{nn} \exp X_n t. \end{aligned}$$

The $n^2 c$ coefficients are not all arbitrary, only n of them being dependent upon the initial conditions. Each column of the c 's is equivalent to the appropriate latent vector, the initial conditions simply determining the factors of proportionality of the latent vectors.

Our task then is to compute the solution of such a set of differential equations; from this solution we can easily identify the appropriate latent vectors. Thus, we reverse the usual procedure in which the latent vectors are first algebraically computed as an aid in giving the solution of the differential equation system. The novelty of the present method consists in the recognition of this fact plus the specification of a speedy method of arriving at a particular solution of the differential equation system. It is fashionable to handle this last problem by means of the Heaviside-Cauchy operational calculus; a careful consideration of these techniques from a computational point of view will show their efficiency to be greatly overrated, involving in this case multiplications of the order n^4 .

It is a commonplace that an n th order differential equation in one

variable can be transformed into n first order equations. It is no less true that a system of the latter form can be converted into single equations of the n th order in each variable; for constant coefficient systems such as the one under consideration, the coefficients of the differential equation are in each case simply the coefficients of the characteristic equation; i.e.,

$$f_n(D)Y_j(t) = 0. \quad (j = 1, \dots, n).$$

If we can identify the appropriate initial conditions for each of the last equations, and then give the solution of each, we should end up with the required c coefficients indicated above. As for the appropriate initial conditions, if $Y(0) = h$, then by repeated use of the original differential equations, it becomes evident that

$$D^i Y(0) = a^i h = h_i.$$

It is a classical fact that the solution of an n th order differential equation for given initial conditions is given by the solution of a set of linear equations whose matrix is of the Vandermonde-Cauchy form

$$\begin{bmatrix} 1 & 1 & \dots & 1 \\ X_1 & X_2 & \dots & X_n \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ X_1^{n-1} & X_2^{n-1} & \dots & X_n^{n-1} \end{bmatrix}$$

the transposed inverse of which is given by

$$\begin{bmatrix} \frac{P_{n-1}(X_1)}{P_n'(X_1)} & \frac{P_{n-1}(X_2)}{P_n'(X_2)} & \dots & \frac{P_{n-1}(X_n)}{P_n'(X_n)} \\ \frac{P_{n-2}(X_1)}{P_n'(X_1)} & \frac{P_{n-2}(X_2)}{P_n'(X_2)} & \dots & \frac{P_{n-2}(X_n)}{P_n'(X_n)} \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ 1 & 1 & \dots & 1 \\ \frac{1}{P_n'(X_1)} & \frac{1}{P_n'(X_2)} & \dots & \frac{1}{P_n'(X_n)} \end{bmatrix}$$

Consequently the n by n c matrix, which is also the matrix made up of columns of latent vectors is given by equation (1) above, where the factors of proportionality $1/P_n'(X_i)$ have been omitted.

When repeated roots are encountered, a generalized Vandermonde determinant is involved whose inversion is easily effected if one simply pursues the close analogy between Vandermonde determinants and expansions in partial fractions.

If the same differential equations are to be solved for many different initial conditions, the above process may be repeated anew. Or a slight economy of effort may be achieved if the inverse of V is worked out once and for all so that the weightings of the different exponential terms can be easily determined by $V^{-1}h$. If the a matrix is symmetrical, the latent vector matrix will be orthogonal so that simple transposition will provide the inverse matrix, except for factors of proportionality.

A SIMPLE METHOD OF INTERPOLATION

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I.—In many branches of statistics it is necessary to determine the coefficients of an n th degree polynomial, $f(x)$, from $n + 1$ observations, $(x_0, f_0; x_1, f_1; \dots; x_n, f_n)$, and to determine readings from this polynomial. For this latter purpose recourse may be had to divided differences, Lagrange's interpolation formula,¹ Aitken's method of interpolation,² etc. However, where a number of readings are to be taken, or where the coefficients are of interest for their own sake, it is necessary to solve a system of linear equations

$$\begin{array}{lcl} a_0 + x_0 a_1 + x_0^2 a_2 + \dots + x_0^n a_n = f_0 \\ \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \text{or } Va = f \\ a_n + x_n a_1 + x_n^2 a_2 + \dots + x_n^n a_n = f_n \end{array}$$

whose matrix is of the familiar Vandermonde form (x_i^j) .

Now in the solution of n th order differential equations with constant coefficients and one-point boundary conditions, such as occur in electrical engineering and other fields of applied mathematics, the solutions can be written in the form of linear combinations of particular solutions, the coefficients being determined by the solution of a transposed Vandermonde set of linear equations.

By means of the Heaviside-Cauchy operational calculus (Laplace transform, etc.), the applied mathematician is able to avoid explicit inversion of such a system of equations. This suggests the possibility of lessening the calculations involved in interpolation by methods analogous to those used in solving differential equations; and upon examination it turns out that the resulting method seems admirably suited to numerical computation, with or without a modern calculating machine.

The amount of effort required in the proposed method may be compared with other methods. To invert the linear equations directly would require multiplications of the order $2n^3/3$ if efficient Gauss-Doolittle methods are used. If advantage is taken of the Vandermonde form of the matrix as in the Aitken method, the calculations of a single reading seem to require multiplications and divisions of the order $3n^2/2$, and, of course, the coefficients of the polynomial are not derived.

The present method entails multiplications of the order of $3n^2$ plus n multiplications for each reading, where the method of synthetic division is used to derive the readings. If two readings are desired, the work is therefore about equal to the Aitken method, and for more than two readings it is very much less.

After using the method for awhile, one will learn how to effect certain economies of labor which need not be indicated here. Thus, if the abscissa points are all positive, as often occurs in practice, the occurrence of negative numbers in the calculations may be lessened by a reversal of signs, followed by a determination of $f(-x)$.

III.—The procedure may be illustrated by a simple numerical example. Suppose we are to fit a quadratic polynomial to the following three points $(-1, 4; 0, 1; 2, 7)$. Clearly the correct answer is given by $f(x) = 2x^2 - x + 1$. To achieve this by the present method, the coefficients of the partial products are computed in the following tabular arrangement.

$$\begin{array}{cccccc} 1 & 1 & 0 & 0 & : & 0 \\ 1 & 1 & 0 & 0 & : & -2 \\ 1 & -1 & -2 & 0 & & \end{array}$$

Each element in a given row is computed from the elements of the preceding row by adding to the element directly above it the product of the element just northwest of it times the number in the preceding row just to the right of the colon. The last row of all gives the coefficients of the auxiliary cubic polynomial.

The second stage of the numerical work consists of dividing this polynomial in turn by $(x + 1)$, x , and $(x - 2)$, so as to calculate the partial remainders. Arranging the work as in synthetic division

$$\begin{array}{cccc|c} 1 & -1 & -2 & 0 & -1 \\ 1 & -2 & 0 & 0 & -1 \\ \hline 1 & -3 & +3 & & \end{array}$$

The second line gives the successive values of $P_1(-1)$. The last recorded item in the third row gives $P_2'(-1)$. If a modern calculating machine is used, only the final element in the third row need be copied.

This process is repeated for each of the roots, without however, having

to recopy the starred first rows. Thus,

$$\begin{array}{cccc|c}
 *1 & -1 & -2 & 0 & 0 \\
 1 & -1 & -2 & 0 & 0 \\
 1 & -1 & -2 & & \\
 *1 & -1 & -2 & 0 & 2 \\
 1 & +1 & 0 & 0 & 2 \\
 1 & 3 & 6 & &
 \end{array}$$

The coefficients in each of the second rows is divided by the last element in the corresponding third row, and when arranged in order give us the inverse of V' .

$$(V')^{-1} = \begin{bmatrix} 1/3 & -2/3 & 0 \\ -1/2 & 1/2 & 1 \\ 1/6 & 1/6 & 0 \end{bmatrix}.$$

Premultiplying this by the row matrix (f_0, f_1, \dots, f_n) , or in this case by $(4, 7, 1)$, the operations described in (1) are carried out, yielding the requisite solution $(2, -1, 1)$.

• Two partial checks on the accuracy of the work may be mentioned. The divisors, being derivatives at simple roots of the auxiliary polynomial, should oscillate in sign, with the last one being positive. Also, the sum of the elements in the last column of the inverse matrix should add up to unity, while the other columns should add up to zero. This provides a check upon all earlier operations. Of course, a final decisive check is provided by evaluating the resulting polynomial to verify that it does go through the prescribed points. This is best done by synthetic division.

IV.—To justify the method it is only necessary to show that the elements of the inverse of the transposed Vandermonde matrix are, in fact, equal to $P_{n-j}(x_i)/P_{n+1}'(x_i)$. This could be done directly by means of contour integration or by the elementary properties of symmetric functions. An indirect proof which lends itself readily to the generalization given below involves the fact that the expression above can easily be shown to give the coefficient of certain terms in the operational solution of differential equation systems. At the same time the classical non-operational solution yields the corresponding coefficients in the form of the inverse of the Vandermonde matrix. Since the classical and operational solutions can easily be shown to be identical, it follows that our theorem must be true.

This suggests a slightly more general method of interpolation in case we are to find a polynomial from a knowledge of its values, and the value of its derivatives up to some varying order, at a number of points. As in the above case, synthetic division, now carried to more rows, is utilized,

and the work of fitting a polynomial of the same degree is only slightly increased.

The matrix of the equations which must be solved now takes the general partitioned form

$$V' = \left[\begin{array}{cccc|cccc|cccc} 1 & 0 & & & 1 & & & & 1 & & & \\ x_0 & 1 & & & x_1 & & & & x_r & & & \\ x_0^2 & 2x_0 & & & x_1^2 & & & & & & & \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & & & & \\ x_0^j & (x_0^j)' & \dots & (x_0^j)^{m_0-1} & x_1^j & \dots & (x_1^j)^{m_1-1} & & x_r^j & \dots & (x_r^j)^{m_r-1} & \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & & & & \\ x_0^n & nx_0^{n-1} & \dots & \frac{n!x_0^{n-m_0+1}}{(n-m_0+1)!} & x_1^n & & & & & & & \end{array} \right]$$

where $(x_s^j)^v = \frac{d^v}{dx^v} [x^j]_{x=x_s}$.

Then by precisely the same kind of proof as that sketched above, it can be shown that the inverse of this generalized Vandermonde matrix is given by

$$(V')^{-1} = \left[\frac{1}{(m_i - k)!k!} \frac{d^{m_i-k}}{dx^{m_i-k}} \left\{ \frac{P_{n-j}(x)}{P_{n+1}(x)/(x-x_i)^{m_i}} \right\}_{x=x_i} \right]$$

where

$$\begin{aligned} \text{where } P_{n+1}(x) &= \sum_{j=0}^r (x-x_j)^{m_j} = x^{n+1} + b_1x^n + \dots + b_{n+1} \\ P_n(x) &= x^n + b_1x^{n-1} + \dots + b_n \\ \text{etc.} \end{aligned}$$

¹ Whittaker, E. T., and Robinson, G., *The Calculus of Observations*, Chap. II, Blackie and Son, Glasgow, 1932.

² Aitken, A. C., "On Interpolation by Iteration of Proportional Parts without the Use of Differences," *Proc. Edinburgh Mathematical Society Series*, 2, No. 3, 56-76 1932.

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